Nocturnal Serum Melatonin Profile in Major Depression in Children and Adolescents

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Background: In major depression, biological rhythm disturbances in sleep, appetite, and mood suggest dysregulation in neuroendocrine functions, possibly in the pineal gland. In this study, pineal gland function was examined by measuring nocturnal serum melatonin levels during both wakefulness and sleep in depressed children and adolescents.

Methods: Twenty-two youths aged 8 to 17 years primarily with major depression were compared with 19 controls. Blood samples were drawn every half hour from 6 PM to 7 AM. Nocturnal serum melatonin levels were measured by radioimmunoassay.

Results: The overall nocturnal serum melatonin profile from 6 PM to 7 AM was significantly higher (mean±SD, 0.18±0.14 nmol/L) in the depressed group than in the controls [mean±SD, 0.15±0.10 nmol/L, $F(1,26)=4.37$, $P<.05$]. In dim light, when the subjects were awake, no difference existed between the 2 groups. After lights-out, from 10 PM to 7 AM, the melatonin profile rose in both groups; however, the depressed group had a significantly higher increase (mean±SD, 0.24±0.14 nmol/L) than the controls [mean±SD, 0.18±0.07 nmol/L, $F(1,26)=4.93$, mean square error=0.11, $P=.04$]. Post hoc analysis showed a significantly higher melatonin profile in depressed subjects without psychosis (n=15) than in depressed subjects with psychosis (n=7) or in the controls.

Conclusion: Measuring the overall nocturnal serum melatonin profile during darkness may help to differentiate children and adolescents with major depression without psychosis from those with psychosis and from controls.

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MOOD DISORDERS such as major depression and dysthymia are serious, episodic, chronic, and debilitating disorders in youth.[1-9] Symptoms of major depression, such as mood, appetite, and sleep disturbances, suggest circadian rhythm disturbances. Because the pineal gland and its hormone, melatonin, contribute to the regulation and entrainment of circadian rhythms to a 24-hour cycle, the study of the pineal gland in depressive disorders may be fertile ground for such investigation.[10,11]

In healthy individuals, nocturnal serum melatonin levels begin to increase 2 hours before bedtime but surge a half hour following lights-out during sleep. Most melatonin is secreted during the dark cycle of the night, from 11 PM to 3 AM, with a 2 AM peak.[12,13] In more than 20 studies on melatonin in adult depression, a number showed a decrease in the nocturnal serum or urine melatonin levels or a phase-shift in the melatonin peak,[14-19] a few showed no difference,[20-23] and 2 showed an increase.[24,25]

To our knowledge, only 3 studies reported on melatonin levels in major depression in youth. Cavallo et al[26] found that the nocturnal serum melatonin level was lower in 9 depressed boys aged 7 to 13 years with mixed diagnoses than in a control group of 10 boys who were of normal height, genetically short, or had delayed pubescence. We measured bedtime and overnight urine melatonin levels in 96 psychiatric inpatients aged 6 to 16 years[27,28] and found that in patients with primary major depression, the overnight urine melatonin level was significantly higher than in patients with secondary depression or in the nondepressed psychiatric controls. Watterman et al[29] reported no difference between the level of 6-hy-

See Subjects and Methods on next page

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SUBJECTS AND METHODS

Children and adolescents aged 8 to 17 years with depressive symptoms who were either inpatients or outpatients and healthy controls were asked to participate in this study with the University of Louisville Human Studies Committee's approval. Assent or informed consent of subjects and a parent or guardian were obtained. Subjects were divided into 2 groups.

SUBJECTS

Depressed Group

Of the 22 patients in this group, 18 were diagnosed with major depression, 1 with severe dysthymia, and 3 with bipolar disorder, depressive phase. Most of the subjects had comorbid diagnoses such as anxiety, conduct and/or oppositional disorder, attention-deficit disorder, or posttraumatic stress disorder.

We included the 1 case of severe dysthymia and the 3 cases of bipolar disorder, depressive phase, with the 18 cases of major depression because longitudinal follow-up of mood disorders in children and adolescents shows overlap and movement between these diagnostic entities. Many dysthymic youth go on to have major depressive episodes, and many youth with major depression, particularly those with psychosis, subsequently develop bipolar disorders.[1,2,30-33]

Control Group

The control group consisted of healthy children and adolescents with no present or past personal or family history of psychiatric disorder, alcohol or other drug abuse, or antisocial behavior in siblings, parents, aunts or uncles, or grandparents. Some of the control subjects were the children of support staff or the nursing staff and others were friends or neighbors of the depressed subjects or controls.

METHODS

Subjects, controls, and parents were interviewed by a member of our research team and the principal investigator. In addition to a clinical interview, the Diagnostic Interview for Children and Adolescents–Revised[34,35] was used, along with the Child Behavioral Checklist, Youth Self-Report[36]; Children's Depression Inventory[37]; Children's Depression Rating Scale[38]; and Depression Self-Rating Scale.[39] Height and weight were measured and stages of pubertal development were obtained using the Tanner stages.[40] From girls, information regarding menarche and date of most recent menstrual period was obtained. None of the subjects had seasonal affective disorder.

Blood samples were drawn from the psychiatric inpatients after the first night of hospitalization. This allowed time to explain the study and receive informed consent. The inpatients went to their rooms after dinner, between 5:30 and 6 PM. The outpatient subjects and the controls, after having dinner, went to the sleep laboratory at the hospital between 5:30 and 6 PM to spend the night. A pediatric nurse introduced a 22-gauge streamline soft venous catheter into a vein. The room lights were dimmed to 50 lux or less (just barely enough light to read) until bedtime at 10 PM. The first blood specimen was collected at 6 PM. Subjects were able to watch television. From 6 PM to 7 AM, intermittent blood sampling was performed every half hour. A blood sample of 3 mL was drawn to have 1 mL of serum for the melatonin assay.

droxymelatonin sulfate in the overnight urine of youth with major depression and that of controls. This study differs from the 2 mentioned above in that the metabolite of melatonin, rather than the parent compound, was measured. Also, urine was collected 12 hours after the administration of insulin as part of a growth hormone challenge study, which might have affected the results.

In the present study, we hypothesized that a dysregulation of the pineal gland in the form of an increase of serum melatonin exists in youth with major depression.[27,28] Recognizing the limitations of previous studies, including ours, we incorporated the following into the research design: a healthy control group; a drug-naive or drug-free period 2 weeks prior to study; extensive diagnostic structured interviews and rating scales; intermittent rather than continuous blood sampling; and dim light during wakefulness to assess dim-light melatonin onset.[11,19]

RESULTS

The depressed group consisted of 9 boys and 13 girls (15 white and 7 African American) and the control group, 11 boys and 8 girls (19 white). Tanner stages 1 and 2 were defined as prepubescence and stages 3 through 5 as pubescence. In both groups, the ratio of pubescent adolescents to prepubescent children was slightly more than 2:1. Concerning the seasonal effect on melatonin levels, depressed subjects were recruited during a depressive episode when they came to the clinic, usually in the spring or fall, whereas control subjects were recruited when they were most available, usually the spring and summer, so there was an uneven distribution of subjects and controls regarding the season of the year. However, in our earlier study,[27,28] neither race nor season affected melatonin levels.

The depressed group weighed significantly more than the control group. A MANOVA was performed on the variables of height and weight in both groups using sex and Tanner stages as independent variables. Both height (r=0.09, P<.01) and weight (r=0.17, P<.01) correlated significantly with serum melatonin levels. Therefore, height and weight variables were used as covariates in subsequent analyses to test effects of the independent variables on serum melatonin levels. The overall serum melatonin level in the depressed group (mean±SD, 0.18±0.14 nmol/L) was significantly higher than in the control group [mean±SD, 0.15±0.10 nmol/L, $F(1,26)$=4.37, mean square error=0.12, P<.05]. Sex, stages of pubescence, and season had no significant effect.

When we eliminated the 1 case of severe dysthy-

Altogether, 27 blood samples were drawn, for a total of 81 mL. All room light was turned off at 10 PM. By collecting samples intermittently from 6 PM onward in dim light or dark conditions, we were able to look for dim-light melatonin onset and possible phase-advanced melatonin secretions. In addition, we examined the patterns of secretion and the peak of melatonin levels throughout the night. The samples were stored at 4°C until transported to the laboratory and were coded to ensure blindness with respect to time of collection and source. Aliquots from coded samples of all subjects were stored frozen at −20°C until assayed. Of a possible 1107 blood samples, 1099 (99.3%) were collected. The other 8 samples (missing data points) were not collected because of vein collapse, infiltration, or other factors.

Melatonin Determinations

Serum melatonin levels were measured using a single-antibody radioimmunoassay, which employed a rabbit antiserum (KALAB, Danville, Calif) and a tritiated melatonin tracer (New England Nuclear, Boston, Mass).[12-14] Samples were extracted with methylene chloride, evaporated to dryness at 37°C, resuspended in assay buffer, and stored at either 4°C if assayed the following day or −20°C if assayed later than the next day. Radioimmunoassays of the extracts were performed to measure melatonin concentrations against a standard curve of known melatonin standard concentrations. Unknown sample extract or assay standard, tracer, and antiserum were mixed together and incubated. After incubation, the bound fraction (that containing the sample melatonin extracts or standards and tracer melatonin bound to antibody) was precipitated with saturated ammonium sulfate and counted in 10 mL of scintillation cocktail in a scintillation counter. Disintegrations per minute of standard divided by disintegrations per minute of the total bound fraction were calculated and plotted on semilogarithmic paper. The disintegrations per minute of unknown divided by disintegrations per minute of the total bound fraction were calculated and results for the unknown samples were read from the curve. Some investigators report melatonin values in nanomoles per liter, some in picograms per milliliter, and some in nanograms per liter. The conversion formula is as follows: picograms per milliliter equals nanograms per liter equals the nanomoles per liter value ×232.26 (molecular weight of melatonin).

Quality Control

The sensitivity of the assay is 0.01 nmol/L and cross-reactivity for most related compounds such as serotonin and 5-hydroxyindoleacetic acid is greater than 1 million to 10 million pg/mL. Within- and between-assay coefficient variations have been less than 5% and 7%, respectively, throughout the anticipated range of melatonin values. Quality control was assured through the use of multiple internal controls for each assay and through regular participation in the College of American Pathologists surveys. The Pediatric Endocrine Laboratory of the University of Louisville is fully licensed to perform a wide range of radioimmunoassay procedures.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS)[41] was used for analysis of data. The multiple analysis of variance (MANOVA) with multiple covariance analysis to correct for height and weight was used at an α level of .05 (2-tailed test). In addition, orthogonal polynomial trend analyses were employed to test for various trends.

mia and the 3 cases of bipolar disorder in the depressive phase, there was no change in the results, which continued to be significant [mean±SD, 0.18±0.15, $F(1,39)=4.34$, mean square error=0.030, $P<.05$].

Figure 1 reveals a generally negatively accelerated trend for melatonin levels for both groups from 6 PM to 7 AM, with a pronounced divergence of the 2 groups after 10 PM, the lights-out time. From 10:30 PM to 7 AM when the lights were out and the subjects and controls were, for the most part, asleep, the melatonin levels in the depressed group (mean±SD, 0.24±0.14 nmol/L) were significantly higher than in the control group [mean±SD, 0.18±0.07 nmol/L, $F(1,26)=4.93$, mean square error=0.11, $P=.04$]. After the lights were out, and eliminating the 4 cases mentioned previously, the mean levels of the depressed group were slightly lower (0.22 nmol/L). The difference continued to be statistically significant. While the lights were on from 6 to 10 PM, there were no differences between the depressed group (mean, 0.08 nmol/L) and the controls (mean, 0.07 nmol/L). These evening levels were somewhat higher than those reported elsewhere, which could possibly be related to the specificity of this radioimmunoassay for melatonin.

There was a significant straight-line relationship between time and melatonin level, overlaid with an appreciable curvilinear trend ($P<.001$).

In post hoc analysis, the depressed group was separated into depressed with psychosis (n=7, mean±SD melatonin level, 0.11±0.11) and depressed without psychosis (n=15, mean±SD melatonin level, 0.19±0.15) (**Figure 2**). The group with psychotic depression had melatonin levels with a trend toward a lower mean compared with controls. The group with nonpsychotic depression continued to show the high melatonin levels seen earlier, with some increase. The estimations of the total melatonin concentrations during the night, or the total area under the curves, are as follows: the depressed group with psychosis, 2.97 nmol/L; the depressed group without psychosis, 5.24 nmol/L; and the control group, 3.94 nmol/L. These differences were significant, especially during the time when the lights were out (MANOVA, $P<.01$ and $P<.001$).

COMMENT

Inherent in the clinical definition of major depression are the symptoms of circadian rhythm disturbances. The suprachiasmatic nuclei of the hypothalamus are the generators of circadian rhythms of slightly more than 24 hours. The pineal gland and melatonin regulate suprachiasmatic nuclei through their receptor sites, entraining circadian rhythms to a 24-hour cycle.[11] Nocturnal

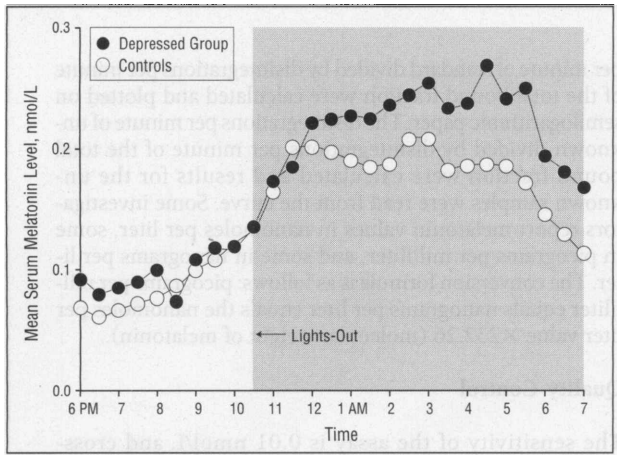

Figure 1. *Nocturnal serum melatonin profile in depressed children and adolescents and healthy controls.*

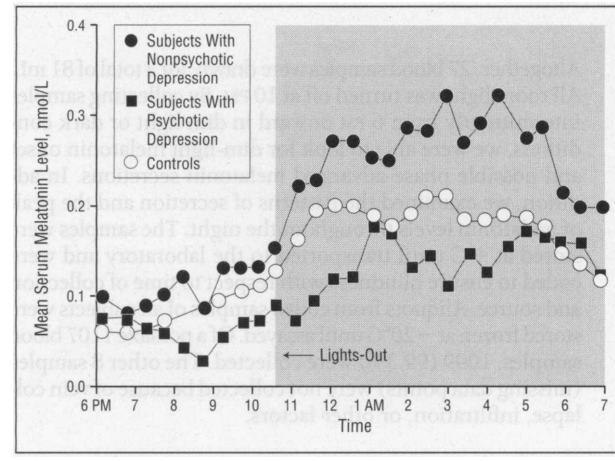

Figure 2. *Nocturnal serum melatonin profile in subjects with nonpsychotic depression, psychotic depression, and healthy controls.*

darkness, primarily between 11 PM and 3 AM, affects the retina, stimulating the β-adrenergic neurons and the suprachiasmatic nuclei, and results in the synthesis of melatonin from serotonin in the pinealocytes of the pineal gland. We measured the nocturnal serum melatonin levels in children and adolescents with major depression and in a healthy control group.

In a study of 22 children and adolescents primarily with major depression, the nocturnal serum melatonin level was significantly higher compared with 19 healthy controls. In post hoc analysis, the depressed patients without psychosis had a much higher nocturnal serum melatonin level than the controls. In contrast, the depressed patients with psychosis had a somewhat lower nocturnal serum melatonin level than the controls.

Our findings are compatible with those of Lewy et al,[42] who found that the plasma melatonin levels in adult patients with manic-depressive illness were lower in the psychotic depressive phase and higher in the manic phase. Recently, Wahlund et al[43] also found that the mean melatonin peak level in depressed adults with psychosis was significantly lower than in those without psychosis.

Interestingly, individuals, whether children, adolescents, or adults, who were depressed with psychosis had lower serum melatonin levels. One wonders if this is just a coincidental finding, whether melatonin may have antipsychotic properties, or, perhaps, whether higher levels of melatonin protect a depressed patient from psychotic symptoms.

Our study had the following limitations: a small number of subjects, mixed diagnostic groups, and comorbid diagnoses. The depressed group, as a whole, weighed more than the controls. Higher levels of melatonin in the depressed group could be related to weight rather than depression. However, Young et al[44] found that there was a reverse relationship between weight, body mass, and serum melatonin levels. If weight had a major effect, the depressed group should have had lower rather than higher melatonin levels.

Looking at the effect of weight on the nocturnal melatonin levels in the 2 subtypes of depression, we found that the mean weight of the depressed group with psychosis was 56.4 kg, and of the depressed group without psychosis, 59.4 kg (SD, ±14.7). There was no significant difference between these 2 groups regarding weight and weight had no effect on their nocturnal melatonin levels.

In most adult studies of major depression, the nocturnal serum melatonin level was lower than in healthy controls, although some studies found no difference and 2 found higher levels. How can these differences be reconciled with each other and with our findings?

During the last 2 decades, the significant advancement in research in child and adolescent depression is related to the use of research diagnostic criteria and depressive rating scales. The criteria for depressive disorders in children and adolescents is basically the same as the criteria for adults. However, biological research findings in depressive disorders in children and adolescents have differed from the findings in adults. For example, in adult major depression, changes in electroencephalographic patterns during sleep, such as an increase of rapid eye movement sleep and a decrease or absence of deep non–rapid eye movement sleep (stages III and IV) are not found in children and adolescents with major depression.

Also, in children and adolescents, in contrast to adults, tricyclic antidepressants are not effective in the treatment of major depression. Is childhood and adolescent major depression biologically different from adult depression even though phenomenologically the same? Or perhaps, childhood and adolescent major depression is similar to that of the 20% to 30% of adults with major depression who are not responsive to tricyclic antidepressants.

Similarity of symptoms within a diagnostic category does not necessarily mean that the underlying biological mechanisms are the same. This is now apparent in, for example, endocrine disorders such as diabetes mellitus or hyperparathyroidism.

Clinical diagnosis of major depression based only on phenomenological symptoms is broad and covers various known and probably unknown subtypes of major depression, whether in adults, children, or adolescents. More attention needs to be given to the diverse biological findings in major depression to decipher biological subtypes of this disorder. Dysregulation of the pineal gland

in the form of increased or decreased nocturnal serum melatonin levels might be related to various subtypes of major depression, rather than invalidity or lack of replicability of various studies.

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RESULTS

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