Biological Psychiatry, Vol. 19, No. 8, 1984

A Chronobiological Study of Melatonin and Cortisol Secretion in Depressed Subjects: Plasma Melatonin, A Biochemical Marker in Major Depression

Bruno Claustrat,[1] Guy Chazot,[2] Jocelyne Brun,[1] Daniel Jordan,[3] and Geneviève Sassolas[4]

Received June 3, 1983; revised January 9, 1984

The temporal organization of plasma melatonin and cortisol secretion was examined in healthy rested controls and in depressed patients: 11 patients suffering from a primary affective disorder (10 female, 1 male) and 8 male controls were studied over a 24-hr period; blood was collected at 2-hr intervals during the day and at 1-hr intervals at night. Plasma melatonin and cortisol levels were determined by radioimmunoassay. In addition, melatonin was determined in plasma sampled at 3 AM in older male controls (n = 8) and in females (n = 10) at ovulation. The controls showed low or undetectable (< 5 pg/ml) diurnal plasma melatonin levels and a very marked nocturnal rhythm (acrophase: 2.27 AM, mesor: 34.4 pg/ml, amplitude: 58.7 pg/ml). For the three control groups, no significant difference was observed in the nocturnal melatonin peak at 3 AM. The depressed patients also showed a significant melatonin rhythm but with lower amplitude (14.5 pg/ml) and mesor (19.1 pg/ml). The latter rhythm was not significantly phase-advanced with respect to the controls (acrophase at 1.18 and 2.34 AM, respectively). In 9 of the 11 patients, nocturnal melatonin secretion was less marked and frequently associated with hypercortisolemia. An additional episodic melatonin secretion was observed in the late afternoon in only two patients. In depressed patients, there was an increase in the mean cortisol secretion level (mesor at 13.6 μg/100

[1]Service de Radiopharmacie et Radioanalyse, Centre de Médecine Nucléaire, Lyon, France.
[2]Unité Neuro-Métabolique, Hôpital Neurologique, Lyon, France.
[3]Laboratoire de Médecine Expérimentale, Faculté de Médecine A. Carrel, Lyon, France.
[4]Unité de Soins, Centre de Médecine Nucléaire, Lyon, France.

0006-3223/84/0800-1215$03.50/1

ml against 9.1 μg/100 ml in the controls), but the amplitude and the acrophase were not significantly modified. These data are discussed in terms of both the hypothalamus-pituitary-adrenal-epiphysis and aminergic abnormalities.

INTRODUCTION

Evidence has been gathered during the past 10 years that the pineal gland controls several important physiological processes. Melatonin, a pineal indoleamine derived from serotonin, shows a circadian variation with high plasma melatonin concentrations at night and low or absent levels during the day. A preliminary report has suggested that a disturbance in the melatonin circadian pattern in conjunction with an abnormality of cortisol secretion can be found in certain depressed patients (Wetterberg *et al.*, 1979). The finding of a disturbed melatonin rhythm has also been emphasized by Mendlewicz *et al.* (1980) and Branchey *et al.* (1982) in a report of four depressed women of whom three were unipolar, and one bipolar. These patients were studied twice, once during the depressed phase and then 4 to 6 weeks later after recovery from the episode of depression. The normal nocturnal increase in plasma melatonin was absent in three of the four patients and no difference in melatonin levels was found before and after treatment in any patient, in contrast to the cortisol rhythm which became normal after recovery. No control subjects were examined. In a more recent study (Beck-Friis *et al.*, 1981), investigation of pineal-pituitary-adrenal relationships in ten patients with major depressive illness showed that patients unresponsive to a dexamethasone test had the lowest plasma melatonin levels. In addition, the ratio of serum cortisol to melatonin at 2 AM was significantly higher in the group of patients having abnormal plasma cortisol suppression when compared with the other groups. These data are indicative of an impaired pineal function together with a pituitary-adrenal disinhibition in patients suffering from major depression. These results need confirmation, however, either by comparing a larger population of depressed patients with healthy controls or by describing in more detail the circadian plasma melatonin pattern; this can be done by drawing more samples over the 24-hr period. Moreover, pathological criteria must be carefully defined because there are physiological variations in the plasma melatonin pattern with age (Iguchi *et al.*, 1982), the seasons (Arendt et al. 1978), and the pituitary-ovarian-cycle (Wetterberg *et al.*, 1976; Birau *et al.*, 1981). In view of these data, we have compared the 24-hr patterns of plasma melatonin and cortisol in depressed and healthy subjects under standardized conditions.

MATERIAL AND METHODS

Subjects and Blood Sampling

Eleven patients (10 female, 1 male, mean age ± SD: 44.5 ± 11.1 years, range 26 to 58 years) suffering from a primary affective disorder and meeting DSM-III criterion E 16 293 3 X were studied during a depressive episode as inpatients at the Centre de Médecine Nucléaire, Hôpital Neurologique, Lyon, France. Clinical data and Hamilton scores for these patients are displayed in Table I. Subjects were free of any somatic disease and had received no drug for at least 10 days prior to the investigation. The women were not taking oral contraceptives; four were menopausal and six were at the follicular phase during the exploration, as attested by plasma estradiol levels. The study was carried out during a depressive episode on the 2nd day after admission. Patients were allowed to rest during the 24-hr period of the test but could sleep only while the lights were off between 10 PM and 7 AM. At 2 PM, an indwelling catheter was inserted and blood samples were drawn over a 24-hr period every 2 hr during the day, and every hour during the night between 10 PM and 6 AM. Heparinized saline was constantly and slowly infused through the system in order to keep the vein open and suspended only immediately prior to each sampling. Blood samples were kept at 4 C until the experiment ended; they were then centrifuged and plasma samples were stored at −20 C until the radioimmunoassay was done.

Under exactly the same conditions as with the experimental subjects, plasma melatonin and cortisol patterns were also determined in healthy young men (25 to 35 years, mean age ± SD: 27.3 ± 3.1 years) in April and May, one of the two periods of the year during which melatonin secretion is believed to be at its lowest (Arendt *et al.*, 1979). In addition, plasma was sampled at the same period of the year at 3 AM in eight older healthy men (56 to 66 years, mean age ± SD: 62 ± 4.2 years) and in eight women (27 to 39 years, mean age ± SD: 34.5 ± 5.1) examined at ovulation for possible *in vitro* fertilization.

Radioimmunoassays

For the melatonin determinations by radioimmunoassay, antimelatonin antiserum and tracer were prepared in our laboratory according to Rollag and Niswender (1976); the specificity of the antiserum has been reported elsewhere (Kopp *et al.*, 1981). We modified the extraction procedure of melatonin from plasma samples using diethyl ether in preference to chloroform. Ether gave a

Table I. Clinical Data and Hamilton Scores for the Depressed Patients

Patient no.	Sex	Age (years)	Diagnosis	Hamilton score	Exploration month	Genital stage	Plasma estradiol[a] pg/ml
1	F	33	Unipolar	45	May	Follicular phase	34
2	F	56	Unipolar	42	February	Menopausal	< 10
3	F	50	Unipolar	30	May	Menopausal	< 10
4	F	51	Unipolar	50	December	Follicular phase	73
5	F	32	Unipolar	42	November	Follicular	16
6	F	43	Unipolar	49	September	Follicular	40
7	M	54	Unipolar	31	November	–	–
8	F	26	Unipolar	42	January	Follicular phase	60
9	F	52	Unipolar	40	April	Menopausal	11
10	F	58	Bipolar	49	April	Menopausal	15
11	F	36	Unipolar	41	March	Follicular phase	65

[a]Normal range: 25 to 100 pg/ml during follicular phase; < 20 pg/ml in menopausal women.

very clear extract, thus ensuring an undetectable blank, and low values (usually less than 5 pg/ml) for plasma melatonin during the light period; 100 pg melatonin added to 1 ml plasma prior to extraction was quantitatively recovered (mean ± 1 SD: 98 ± 3 mg/ml, $n = 20$). The same plasma melatonin concentration showed within- and interassay coefficients of variation, respectively, of 4 and 10.5%. Sensitivity of the method was routinely 5 pg melatonin per ml of plasma. Plasma cortisol levels were determined on diluted samples by radioimmunoassay using an antiserum raised in rabbit immunized with cortisol 3-0 (carboxymethyl oxime) bovine serum albumin conjugate and ^{125}I-cortisol (Clinical Assay Travenol Laboratory, Cambridge, MA) as tracer. The within- and interassay coefficients of variation were less than 3.5 and 5%, respectively, at 10 μg/100 ml cortisol level.

Statistical Analysis

The data were analyzed according to a program devised for rhythm analysis. This program, based on Fourier analysis, was used to fit the experimental curve of the plasma variations in each hormone to a sinusoidal function. The program provided the mean hormonal value (mesor) and an estimation of the amplitude and the acrophase of the resulting sinusoidal function (Anderson, 1971; Roussel *et al.*, 1976). In addition, the mean levels for plasma cortisol ($\bar{F}$) and melatonin ($\bar{M}$) over 24 hr were determined for each patient, thus enabling the ratio of plasma cortisol to melatonin to be calculated as an index of the pituitary-adrenal-pineal balance. Mean levels for light and dark period plasma melatonin ($\bar{M}1$ and $\bar{M}d$, respectively) were also evaluated. Results were expressed in terms of pg/ml for $\bar{M}$, $\bar{M}1$, $\bar{M}d$, and in terms of μg/100 ml for $\bar{F}$.

RESULTS

The eight younger control subjects had very low or undetectable plasma melatonin levels – less than 5 pg/ml – during the light period between 10 AM and 6 PM. Melatonin circadian rhythm was very marked and mean nocturnal levels plateaued between 11 PM and 4 AM (Fig. 1). Chronobiological analysis gave an amplitude of 58.7 pg/ml and a mesor of 34.4 pg/ml; the acrophase was located at 2.27 AM (Table II, Fig. 2). In addition, plasma melatonin peaks observed at 3 AM for the women at ovulation (72.9 ± 26 pg/ml, mean ± SD) and for the older control subjects (84.7 ± 20 pg/ml) were similar to those of the younger controls (86.5 ± 22 pg/ml). In the depressives, a significant but less marked melatonin rhythm was detected, the amplitude and the mesor being 14.5 and 19.1 pg/ml, respectively. This rhythm was not significantly phase-advanced (Table II, Fig. 2). This overall reduction of melatonin secretion in

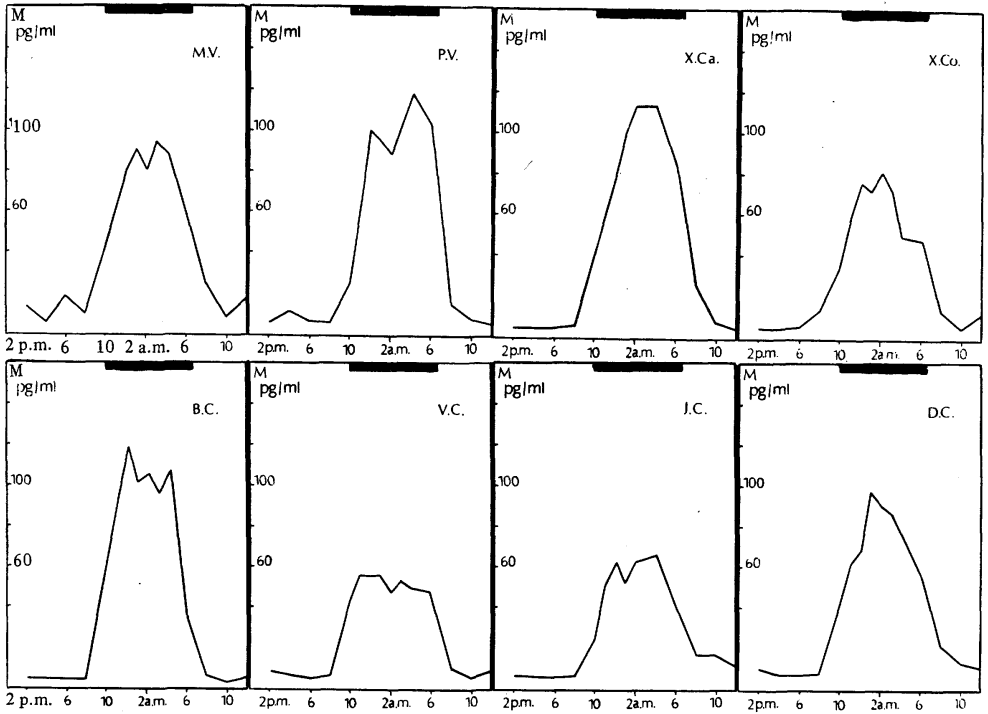

Fig. 1. Individual 24-hr profiles of plasma melatonin in control subjects.

Table II. Chronobiological Parameters for Melatonin and Cortisol Secretion in Control and Depressed Subjects

	Melatonin			Cortisol		
Group	Mesor pg/ml	Amplitude[a] pg/ml	Acrophase[a] hr:min	Mesor μg/100 ml	Amplitude[a] μg/100 ml	Acrophase[a] μg/100 ml
Controls	34.4	58.7 ± 6	2:27 ± 0:24	9.1	6.3 ± 1.2	8:40 ± 0:44
Depressives	19.1	14.5 ± 7.8	1:18 ± 2	13.7	6.1 ± 2.4	9:55 ± 1:33

[a]95% confidence intervals.

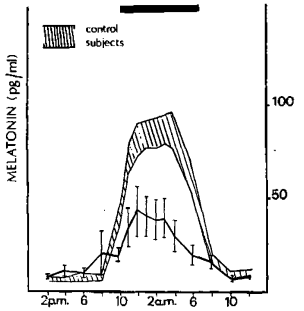

Fig. 2. Plasma melatonin variations (mean ± SEM) over a 24-hr period in controls and depressed patients.

the depressed patients group was a consequence of a lower nocturnal secretion: $\bar{M}1$ was not significantly modified, but $\bar{M}d$ was 32.7 ± 29.4 pg/ml in the depressed patients *vs.* 73.8 ± 17.4 pg/ml in the control group ($p < 0.01$) (Table III). In addition, the plasma melatonin peak at 3 AM for depressives was decreased when compared to that of ovulating women (39.3 ± 38.4 *vs.* 72.9 ± 26 pg/ml, $p < 0.05$). In contrast, cortisol secretion was increased in depressed patients as expressed by the mesor but the amplitude and the acrophase were not significantly different from control values (Table II, Fig. 3).

The values of $\bar{M}$ and $\bar{F}$ in the depressed group were significantly different from the corresponding values in the control group ($p < 0.02$) and the $\bar{M}/\bar{F}$ ratios were also significantly different for the two groups ($p < 0.001$) (Table III). This ratio, however, was within the normal range for two patients (Fig. 4). Considering the individual hormonal profiles, all depressed patients maintained a significant melatonin circadian rhythm with a maximum nocturnal level

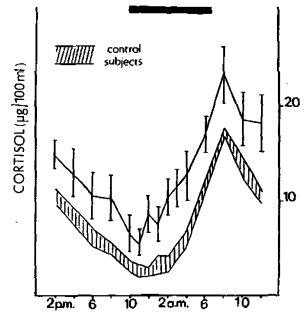

Fig. 3. Transverse mean ± SEM of the 24-hr profiles of plasma cortisol obtained in depressed patients and controls.

Table III. Mean Levels of 24-Hour Plasma Cortisol ($\bar{F}$) and 24-Hour-Plasma Melatonin ($\bar{M}$); Light Period Plasma Melatonin ($\bar{M}l$), Dark Period Plasma Melatonin ($\bar{M}d$), and Plasma Cortisol:Melatonin Ratio ($\bar{M}/\bar{F}$) in Control and Depressed Subjects

	$\bar{M}$ (pg/ml)	$\bar{M}1$ (pg/ml)	$\bar{M}d$ (pg/ml)	$\bar{F}$ (μg/100 ml)	$\bar{M}/\bar{F} \times 10^4$
Controls mean	41.2	9.4	73.8	7.4	5.9
± 1 SD	± 9.5	± 2.6	± 17.4	± 1.3	± 1.4
Lower limit (mean − 2 S.D.)	22.1		39.0		3.0
Upper limit (mean + 2 S.D.)				10.2	
Depressives 1	23.4	8.8	36.1	9.8	2.3
2	39.5	20	56.6	7.2	5.4
3	17.0	13.1	20.5	12.6	1.3
4	9.0	5	12.6	16.9	0.5
5	10.5	9	11.8	11.8	0.8
6	10.8	5.8	15.1	26.1	0.4
7	11.2	5	16.6	7.9	1.4
8	7.6	6	9.0	10	0.7
9	13.5	4.2	21.6	8.0	1.6
10	34.2	11.4	54.2	12.4	2.7
11	71	31	106	19.6	5.4
Mean	22.5[a]	10.8[b]	32.7[b]	12.9[a]	2.0[c]
± 1 SD	± 19.2	± 8.1	± 29.4	± 5.7	± 1.8

[a] $p < 0.02$.
[b] $p < 0.01$.
[c] $p < 0.001$.
[d] Nonsignificant when values for controls and depressives are compared, using Student's t test.

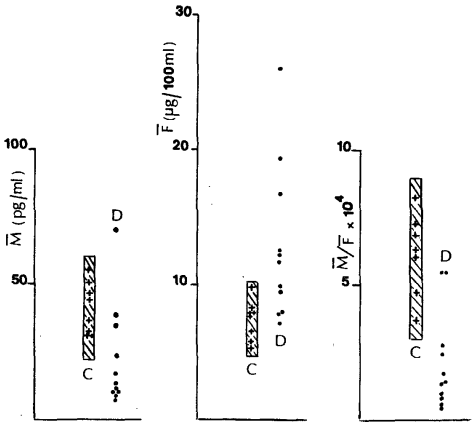

Fig. 4. The 24-hr mean plasma level for cortisol ($\bar{F}$) and melatonin ($\bar{M}$), and $\bar{M}/\bar{F}$ ratio in depressed patients (D) and controls (C). Hatched areas correspond to mean ± 2 SD for controls.

which was in all cases at least triple the diurnal base level (Fig. 5). Two patients (1 and 2) demonstrated quantitatively normal mean cortisol and melatonin levels, but there was evidence of nocturnal hypercortisolemia in patient 1, and a small additional melatonin peak was noted in patient 2 at 4 PM. Four patients (3, 4, 5, and 6) showed hypercortisolemia in combination with a marked reduction in the melatonin rhythm. In three patients (7, 8, and 9) there was a hyposecretion of melatonin superimposed on a cortisol level that was within normal range for the 24 hr mean but with variations in rhythm. Patient 10 demonstrated a normal melatonin rhythm and a slightly increased cortisol level at 8 AM; and patient 11 showed an increased melatonin level in addition to a hypersecretion of cortisol.

DISCUSSION

Our data show a marked decrease in melatonin secretion in major depression. Plasma melatonin levels were compared between groups of healthy young adult males and depressed patients (10 female, 1 male) and between ovulating women and depressives. In addition, a 3 AM plasma melatonin peak was determined in a group of older controls. The choice of these control subjects is justified because: (i) Although age-related variations in melatonin secretion have

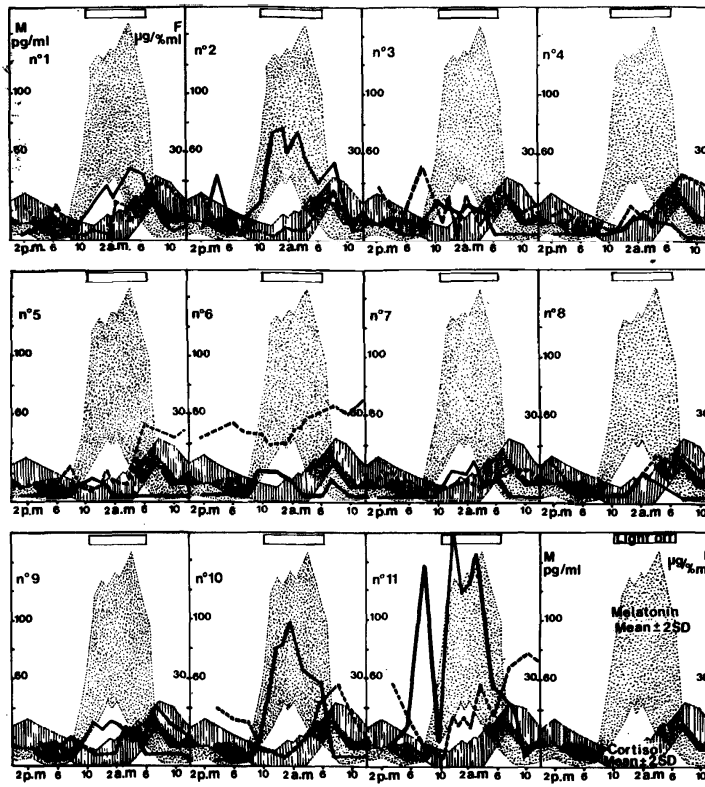

Fig. 5. Individual 24-hr profiles of melatonin (solid line) and cortisol (dotted line) in depressed patients. The hatched area represents the normal range (mean ± 2 SD) for cortisol, and the dotted area the normal range for melatonin.

been reported (Iguchi *et al.*, 1982) with a reduction in nocturnal plasma levels in a group of very old subjects (mean age, 84), our study performed with the eight older control subjects (mean age 62) indicated little difference in melatonin secretion when compared with the younger controls. (ii) The circadian rhythm of melatonin secretion in healthy young women is modulated by hypothalamic-pituitary-ovarian activity, with a decrease in the melatonin nocturnal peak at the time of ovulation (Birau *et al.*, 1981), but no depressed female subject was at this menstrual phase during this study. In addition, we have

shown that in women examined at ovulation the melatonin peak was similar to that observed in healthy young men at the same period of the year.

The very low diurnal plasma melatonin levels (less than 10 pg/ml) reported here do not agree with those found by other authors, who have used the Rollag and Niswender methodology for melatonin radioimmunoassay, but are similar to those measured by radioimmunoassay using [^{3}H]-melatonin as tracer (Arendt *et al.*, 1979) or by gas chromatography-negative chemical ionization mass spectrometry (Lewy and Newsome, 1978). The discrepancy in the radioimmunoassays is apparently a consequence of the modification of the melatonin extraction procedure since diethylether was used here instead of chloroform prior to radioimmunoassay. The latter solvent may be a source of blank and could therefore dramatically increase plasma levels.

Results from the present study show that a melatonin hyposecretion occurs in depressed patients as assessed by several parameters, $\overline{M}$, $\overline{Mn}$, and the $\overline{M}/\overline{F}$ ratio, which were significantly decreased, as well as the reduced amplitude and mesor of the circadian rhythm of plasma melatonin. This melatonin rhythm remains significant however in all depressives. Melatonin secretion takes place principally during the night, though the pattern in two patients implies a supplementary peak in the late afternoon. Chronobiological analysis indicated that the acrophase occurred at 1:18 PM for the depressed patients and at 2:34 PM for the controls, but this difference is not significant.

On the basis of the brain amine deficiency concept of depression, the flattened melatonin secretion rhythm can be considered a result of concomitant disturbances of both serotoninergic and noradrenergic systems: (i) The pineal gland can synthesize melatonin from systemic tryptophan; all the enzymes including tryptophan hydroxylase are present in the epiphysis. In addition, serotonin can act as a precursor after uptake by pinealocytes (Ducis and Distefano, 1980). Consequently, a tryptophan deficiency results in a decrease in brain serotonin and melatonin concentrations, and this is more likely to occur in depressives as nocturnal hypercortisolemia can induce tryptophan hypercatabolism after activation of pyrrolase (Curzon and Green, 1968).

(ii) The neurotransmitter norephinephrine, released from sympathetic endings, activates β-adrenergic receptors on the pinealocytes and stimulates the synthesis of *N*-acetyltransferase, the enzyme which plays a major role in the regulation of the melatonin rhythm. Thus, although the suggestion that a reduction in the availability of synaptic noradrenalin might be a cause of depression remains unproven (Schildkraut, 1965), melatonin hyposecretion could be supportive evidence for this hypothesis. Impaired melatonin secretion was frequently associated in our patients with hypercortisolemia, but these two biological parameters were not statistically correlated ($R = 0.15$, $0.5 < p < 0.9$). This is not surprising however, because evidence is now available supporting the view that the pineal gland participates in the regulation of the

hypothalamic-pituitary-adrenal axis; pinealectomy produces adrenal hypertrophy (Vaughan *et al.*, 1972), and inversely, several studies have demonstrated a hyperactivity of the pineal gland following exposure to stress (Miline, 1980). In addition, corticosteroids can modify the density of β receptors, as has been demonstrated for blood cells (Davies and Lefkowicz, 1980). Nevertheless the responsiveness of β receptors in a hypercortisolemic situation might depend on whether corticosteroid levels are acutely increased as in response to stress, or chronically modified as in major depression. In the latter situation a subsensitivity of the pineal β receptors would be a likely result.

The overall conclusion from this study is therefore that major depression could be correlated with concomitant dysregulation of several neuroendocrine systems, among which the absence of the "tranquillizing" role of the pineal gland (Romijn, 1978) would play a particularly important role. Plasma melatonin determination, under these circumstances, could be of primary interest as a marker.

RERERENCES

Andreason, T. W. (1971). In *Time Series Analysis,* Wiley, New York, p. 92.

Arendt, J., Wirz-Justice, A., Bradtke, J., and Kornemark, M. (1979). Long term studies on immunoreactive human melatonin. *Ann. Clin. Biochem.* 16: 307.

Beck-Friis, J., Aperia, B., Kjellman, B. F., Ljunggren, J. G., Petterson, U., Sjölin, A., Tham, A., Unden, F., and Wetterberg, L. (1981). Hormonal changes in acute depression. Abstract from the 3rd World Congress of Biological Psychiatry, Stockolm, Sweden, June 28-July 3.

Birau, N., Birau, M., and Schlott, W. (1981). Melatonin rhythms in human serum, in *Advances in the Biosciences: Melatonin – Current States and Perspectives,* Birau, N., and Schloot, W. (eds.), Pergamon Press, New York, pp. 287–295.

Branchey, L., Weinberg, U., Branchey, M., Linkowski, P., and Mendlewicz, J. (1982). Simultaneous study of 24-hour patterns of melatonin and cortisol secretion in depressed patients. *Neuropsychobiology* 8: 225.

Curzon, G., and Green, A. R. (1968). Effect of hydrocortisone on rat brain 5-hydroxytryptamine. *Life Sci.* 7: 657.

Davies, A. O., and Lefkowicz, R. J. (1980). Corticosteroid-induced differential regulation of β adrenergic receptors in circulating human polymorphonuclear leukocytes and mononuclear leukocytes. *J. Clin. Endocrinol. Metab.* 51: 599.

Ducis, I., and Distefano, V. (1980). Evidence for a serotonin uptake system in isolated bovine pinealocyte suspensions. *Mol. Pharm.* 18: 438.

Iguchi, H., Kato, K. I., and Ibayashi, H. (1982). Age-dependent reduction in serum melatonin concentrations in healthy human subjects. *J. Clin. Endocrinol. Metab.* 55: 27.

Kopp, N., Claustrat, B., and Tappaz, M. (1981). Evidence for the presence of melatonin in the human brain. *Neurosci. Lett.* 19: 237.

Lewy, A. J., and Newsome, D. A. (1983). Different types of melatonin circadian secretory rhythms in some blind subjects. *J. Clin. Endocrinol. Metab.* 56: 1103.

Mendlewicz, J., Branchey, L., Weinberg, U., Branchey, M., Linkowski, P., and Weitzmann, E. D. (1980). The 24-hour pattern of plasma melatonin in depressed patients before and after treatment. *Commun. Psychopharmacol.* 4: 49.

Miline, R. (1980). The role of pineal gland in stress. *J. Neurol. Transm.* 47: 191.

Rollag, M. D., and Niswender, G. D. (1976). Radioimmunoassay of serum concentration of melatonin in sheep exposed to different lighting regimens. *Endocrinology* 98: 482.

Romijn, H. J. (1978). The pineal, a tranquillizing organ? *Life Sci.* 2257.

Roussel, B., Chouvet, G., and Debilly, G. (1976). Rythmes circadiens des temperatures internes et ambiance thermique chez le rat. *Pflügers Arch. Pharmakol.* 365: 183.

Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: A review of supporting evidence. *Ann. J. Psychiat.* 122: 509.

Vaughan, M. K., Vaughan, G. M., Reiter, R. J., and Benson, B. (1972). Effect of melatonin and other pineal indoles on adrenal enlargement produced in male and female mice by pinealectomy, unilateral adrenalectomy, castration and cold stress. *Neuroendocrinology* 10: 139.

Wetterberg, L., Arendt, J., Paunier, L., Sizonenko, P. C., Vandonselaar, W., and Heyden T. (1976). Human serum melatonin changes during the menstrual cycle. *J. Clin. Endocrinol. Metab.* 42: 185.

Wetterberg, L., Beck-Friis, J., Aperia, B., and Petterson, U. (1979). Melatonin/cortisol ratio in depression. *Lancet* 2: 1361.