Patterns of Melatonin Rhythms in Depression

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With 6 Figures

Summary

The nocturnal rise of melatonin in serum of humans is the result of
endogenously released norepinephrine (NE) acting upon beta-adrenergic
receptors of the pineal gland. As there is much interest in the possibility of
there being changes in the function of beta-receptors in depressed patients,
the nocturnal rise of melatonin was measured in depressives and healthy
control subjects. In one study, multiple serum samples were taken between
4.30 p.m. to 7.30 a.m. in seven male depressed patients with melancholia
and five healthy male control subjects. The melancholic patients had a
significantly reduced nocturnal elevation of melatonin. In a separate study,
serum samples were taken at 8 a.m. and 11 p.m. in melancholic depressives,
non-melancholic depressives and healthy control subjects. The melancholic
patients had a significantly lower concentration of melatonin at 11 p.m., but
not at 9 a.m., than that measured in either the control subjects or the non-
melancholic depressed patients. These results are similar to those found
recently by several other groups of investigators. Further research is indicat-
ed to elucidate mechanism(s) responsible for this phenomenon.

Key words: Melatonin, depression, antidepressants, beta-adrenoceptors.

Introduction

For many years now, central noradrenergic neurons have been
speculated to be involved either in the etiology of some of the signs

or symptoms of major depressive disorders and/or the mechanism of action of antidepressant drugs (see *Prange*, 1964; *Schildkraut*, 1965; *Frazer* and *Conway*, 1984). Most of the data used to support this idea was generated preclinically—from studies demonstrating that anti-depressants had robust effects on central noradrenergic neurons. Since then, a variety of strategies have been employed in an attempt to detect either alterations in noradrenergic neuronal activity or effects of antidepressants on noradrenergic neurons in depressed patients. Although pronounced effects of many types of anti-depressants on the function of noradrenergic neurons has emerged (*Linnoila et al.*, 1982; *Veith et al.*, 1983; *Ross et al.*, 1985; *Bowden et al.*, 1985) no uniform alteration of noradrenergic neurons has been found in depressives. In earlier studies, data obtained was interpreted in favor of underactive functioning of noradrenergic neurons (*Maas et al.*, 1968; *Beckman* and *Goodwin*, 1980); more recently evidence in favor of excessive activity of these neurons in depressed patients has been obtained (*Koslow et al.*, 1983).

To date, the measurement of norepinephrine (NE) or its metab-olites in some bodily fluid has been the strategy used by most investigators studying this issue. An alternative strategy would be to measure in depressed patients responses due to activation of nor-adrenergic receptors. Similarly, the effect of treatment of depressives with antidepressants on NE—elicited responses could be studied. As current views of antidepressant drug action (as well as ideas of altera-tions of noradrenergic activity in depression) center around possible changes in receptor function (*Frazer et al.*, 1974; *Vetulani* and *Sulser*, 1975; see *Charney et al.*, 1981; see *Frazer*, 1981) employment of such a strategy could be illuminating.

This strategy can be used in depressives by measuring the noctur-nal rise in serum of 5-hydroxy-N-actyl-serotonin (melatonin) secret-ed from the pineal gland. Humans as well as other animals show a cir-cadian rhythm of melatonin in serum with highest concentrations being found at nighttime (*Kennaway et al.*, 1977; *Arendt et al.*, 1977). In animals, the signal for this rhythm originates in the suprachiasmat-ic nucleus (*Moore*, 1978) and is inhibited by environmental lighting (see *Lewy*, 1981). Light impinging on the retina acts *via* a multisynap-tic pathway to inhibit the firing of the superior cervical ganglia which results in diminished release of NE from the sympathetic nerves innervating the pineal (*Brownstein* and *Axelrod*, 1974). Upon release, NE activates beta-adrenergic receptors on the pinealocyte resulting in increased synthesis of melatonin (see *Axelrod*, 1978). The regula-tion of melatonin synthesis may be similar in humans to that just described in rats; nocturnal melatonin levels in man decrease

markedly after treatment with beta-adrenergic antagonists like propranolol or atenolol (*Hanssen et al.,* 1977; *Moore et al.,* 1979; *Cowen et al.,* 1983 b). Also, the nocturnal rise of melatonin is absent in patients with transection of the cervical spinal cord (*Kneisley et al.,* 1978). From such data it may be inferred that in humans the synthesis and release of melatonin from the pineal gland is regulated by NE acting on pineal beta-adrenergic receptors. Given this, it is of interest that administration of the beta-adrenergic agonist isoproterenol to humans does not raise the concentration of melatonin in serum (*Vaughan et al.,* 1976; *Moore et al.,* 1979) as it does in the serum of the rat (*Lewy,* 1981; *Heydorn et al.,* 1982). The reason for the negative result with isoproterenol in humans is not known. It may be due to the need to use doses of the drug that would cause unacceptable cardiovascular effects. Whatever the reason, one cannot use isoproterenol to stimulate melatonin in humans. Nevertheless, for reasons enumerated above, the nocturnal rise of melatonin in serum of humans does appear to be a consequence of endogenously released NE acting upon pineal beta-adrenergic receptors. Given the marked advances in techniques available to measure melatonin accurately in the serum of humans (discussed elsewhere in this volume), we have measured the nocturnal rise of melatonin in depressed patients, both before and during treatment with the tricyclic antidepressant, desmethylimipramine (DMI), as a measure of noradrenergic responsiveness in depressed patients.

Nocturnal Melatonin Secretion in Depressed Patients and Control Subjects

Philadelphia Study

Seven male patients [age 42 ± 4 years ($\overline{X} \pm$ S.E.M.)] who met DSM III criteria for Major Depressive Disorder, melancholic subtype, and five male healthy control subjects (age 32 ± 5 years) were studied. The patients were all moderately to severely depressed, required hospitalization, and had a mean Hamilton Rating Scale for Depression score of 31 ± 3.4 (*Hamilton,* 1960). The weights of the patients (77 ± 3.4 kg) and the control subjects (78 ± 2.5 kg) were not significantly different. The patients were drug-free for at least 7 days prior to testing and had received no antidepressants within 2 weeks of testing. Blood was sampled for subsequent melatonin determination at 4.30 p.m., 6 p.m., 7.30 p.m., 9 p.m., 10.30 p.m., 12 p.m., 1.30 a.m., 3 a.m., 4.30 a.m., 6 a.m. and 7.30 a.m. Lights were off from 10 p.m. to 6.30 a.m. Serum was frozen and assayed within two

months using the radioimmunoassay procedure developed by *Rollag* and *Niswender* (1976). Further details of this study and the assay have been given previously (*Brown et al.*, 1985).

The results of this study are summarized in Fig. 1. The concentration of melatonin in serum was less in all subjects when sampled during light than it was during the nighttime. Dark phase concentrations of melatonin in serum rose significantly in all study groups, as
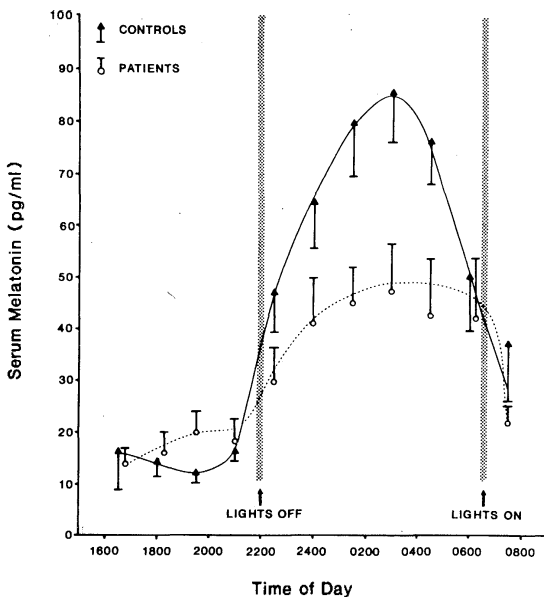

Fig. 1. Serum concentrations of melatonin in male control subjects (N = 5) and male melancholic depressives (N = 7) measured at multiple times during the day and night. Each point and bracket represents the mean value of melatonin plus or minus the S.E.M. Both groups of subjects showed a rise in melatonin after the lights were turned off at 10 p.m., as revealed by a significant effect of time in a two-way ANOVA (F = 23.9, p < 0.001). The significant interaction term in the ANOVA (F = 3.8, p < 0.001) indicated that the rise of melatonin over time was different in the patient and control groups. Inspection of the data shows a reduced nocturnal rise in the melancholic depressives (from *Brown et al.*, 1985)

revealed by analysis of variance on time of sampling ($F = 23.9$, $df = 10$, $p < 0.001$). However, depressed patients with melancholia experienced a less marked increase as indicated by the significant interaction between diagnosis and time ($F = 3.8$, $df = 10$, $p < 0.001$). Post hoc analyses revealed no significant differences between patients and control subjects at any individual time point. However, the peak values of melatonin for each individual was less in the melancholic patients (53 ± 10 pg/ml) than that measured in the control group (89 ± 9 pg/ml) ($p < 0.05$, $df = 10$, t-test). No significant correlation was found between age and the peak value of melatonin measured in either melancholic patients (*Spearman* $r = 0.3$) or healthy control subjects (*Spearman* $r = 0$).

In addition to analyzing the magnitude of the nocturnal rise of melatonin in the serum of the patients and control subjects, separate statistical analyses were done to determine if there was a shift in the time at which the peak concentrations occurred. Inspection of Fig. 1 suggests that control and patient groups reached peak values of melatonin at the same time (approximately 3 a.m.). Examination of the data from individual patients, though, indicated considerably more variability in the time at which peak values occurred among patients. There are several ways to confirm and express this difference. The F_{max} test for equality of variances may be applied. Taking the time at which 90% of the peak level is first reached (to reduce effect of fluctuations about the peak) where 12 p.m. is 0, 1.30 a.m. is $+ 1.5$, 10.30 p.m. is $- 1.5$, the mean and SD for controls was 1.8 ± 0.67 and for patients was 1.5 ± 2.1. The F-test for homogeneity of variances equals 9.82 ($df = 4$, 6; $p < 0.01$).

Trend analyses were performed also, using ANOVA, to determine how well patient and control group data could be approximated by linear and quadratic equations determined separately for each group. For the control group, the quadratic term achieved an $F = 40.1$, $p < 0.00001$, but for the patient group $F = 8.6$, $p < 0.01$. Whereas the control group significantly deviated from linear ($F = 7.3$, $p < 0.001$), the patient group did not ($F = 1.6$).

New York Study

In this study, three different groups of subjects were included: a) hospitalized—patients with a DSM III diagnosis of Major Depressive Disorder, melancholic subtype (14 females, 5 males; age 62 ± 2.5 years); b) hospitalized—patients with a diagnosis of Major Depressive Disorder without melancholia (9 females; age 57 ± 4.5 years) and c) healthy controls (7 males; age 41 ± 4.0 years). The melancholic

patients were more severely depressed (Hamilton Score, 36 ± 4.3) than the non-melancholic patients were (Hamilton Score, 23 ± 1.7; $p < 0.001$). Lights were off from 10 p.m. to 6.30 a.m. and blood for melatonin was sampled at 11 p.m. and 9 a.m. Serum was frozen and shipped to Philadelphia every two months for assay in the same laboratory and by the same method as described above.

Because there are previous reports of an association between reduced nocturnal secretion of melatonin in depressives and abnormalities in hypothalamic–pituitary-adrenal cortical (HYPAC) activity (*Wetterberg,* 1983), 16 melancholic and seven non-melancholic depressed patients in New York also underwent 1mg dexamethasone supression tests (DST's) within three days of the melatonin study. DST non-suppression was defined as a plasma cortisol value above $5 \mu g/dl$ at 8 a.m. or 4 p.m. on the day following dexamethasone administration. Cortisol was determined by radioimmunoassay (*Stokes et al.,* 1984).

The results obtained in New York City for melatonin at 11 p.m. are shown in Fig. 2. Because values obtained in male depressed patients with melancholia (40 ± 10.8 pg/ml) were not significantly different from those measured in female depressed patients with melancholia (35 ± 5.1 pg/ml; $p > 0.6$, Mann-Whitney test), results from male and female melancholic patients were combined. The concentration of melatonin in serum measured in the melancholic patients (36.4 ± 4.6 pg/ml) was significantly lower than that determined in either control subjects (60.3 ± 8.0 pg/ml; $p < 0.05$, Mann-Whitney test) or non-melancholic depressed patients (58.6 ± 9.6 pg/ml, $p < 0.05$). Because all nine non-melancholic depressed patients were female, their melatonin values (58.6 ± 9.6 pg/ml) were compared with those of the female melancholic depressed patients (35 ± 5.1 pg/ml); a significant difference was found ($p < 0.05$, Mann-Whitney test).

No significant differences in 9 a.m. serum melatonin values were found among melancholic depressed patients (14.0 ± 2.0 pg/ml), non-melancholic depressed patients (15.1 ± 2.3 pg/ml), or control subjects (14.0 ± 2.9 pg/ml).

There were no significant correlations between melatonin measured at 11 p.m. and age for melancholic patients, control subjects, non-melancholic patients, or the entire sample (Spearman r ranged from -0.1 to 0.6). The Pearson correlation coefficient for lognormalized distributions of melatonin levels at 11 p.m. and weights in all depressed patients was 0.12 (NS). Thirteen of 16 melancholic patients given the DST were nonsuppressors; in contrast, of six melancholic patients, only one was a nonsuppressor ($p = 0.05$,
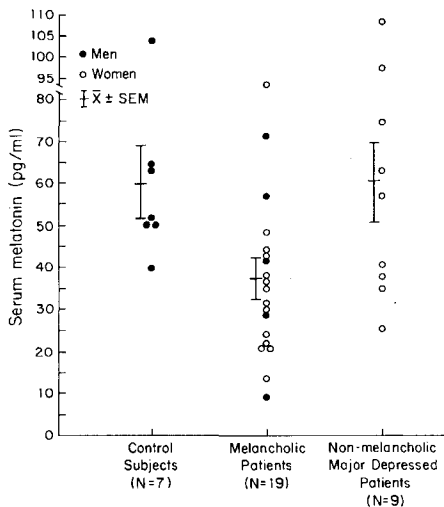
Fig. 2. The concentration of melatonin in serum of control subjects (N = 7), melancholic depressed patients (N = 19) and nonmelancholic depressives (N = 9). Blood samples were obtained during the night, at 11 p.m. The range shown is mean ± S.E.M. The mean value obtained in melancholic patients is significantly lower than that measured in either control subjects or non-melancholic depressives (from *Brown et al.*, 1985)

Fishers exact test). However, values of melatonin measured at 11 p.m. in non-suppressors (41.3 ± 6.2 pg/ml) and suppressors (44.7 ± 7.4 pg/ml) were not significantly different (p > 0.7, Mann-Whitney test).

The major finding of these studies was lower nocturnal concentrations of melatonin in serum in patients with major depressive disorder, melancholic subtype, compared with that measured either in healthy control subjects or patients with major depression without melancholia. In Philadelphia, both study groups were male and the depressed patients were all diagnosed as melancholic. Results support existence of decreased nocturnal melatonin in melancholic males compared to male healthy controls. The New York study confirms and extends the findings from Philadelphia. A melancolic sample comprised of both sexes was found to have lower 11 p.m. melatonin concentrations than either a male healthy control group or a female

non-melancholic depressed sample. The values obtained in the non-melancholic patients appear comparable to those obtained in male healthy controls. The search for an association between reduced nocturnal melatonin secretion and increased adrenocortical activity (as marked by a non-suppressing DST) proved negative, although the numbers of subjects studied was relatively small. This possible association deserves more investigation in future studies.

Our study was not the first to measure melatonin in depressives. *Jimerson* and coworkers (1977) measured urinary melatonin levels in six patients with primary affective disorder and in six control subjects. They were unable to demonstrate a significant difference using a relatively insensitive tadpole bioassay. Also, most melatonin is excreted in the urine as the sulfatoxy-conjugate of 6-hydroxymelatonin and not as free melatonin (*Kopin et al.,* 1961; *Jones et al.,* 1961; *Sisak et al.,* 1979). *Lewy* and associates (1979) reported that melatonin levels in serum were higher in bipolar patients in their manic than in their depressed phases. *Wetterberg et al.* (1979) found lower melatonin in serum at midnight and 2 a.m. but not at 6. a.m. in a patient during one of many suicidal, depressed episodes. *Mendlewicz* and colleagues (1979) found lowered amplitude of the nocturnal rise in melatonin in three of four severely depressed women during depression and again four to six weeks later after recovery. However, since they reported very high daytime concentrations of melatonin in serum—around 100 pg/ml—the accuracy of the assay used in the study by *Mendlewicz et al.* (1979) is questionable. *Wirz-Justice* and *Arendt* (1979) found lower melatonin levels in serum of six bipolar depressed patients at 8 a.m. compared to concentrations found in twelve healthy controls.

Recently, *Wetterberg* (1983) summarized a series of studies by his group and described lower melatonin in serum at 2 a.m. in 17 major depressed patients who were nonsuppressors to dexamethasone, as compared to that measured in either 22 controls or 15 major depressed patients who had normal suppression of cortisol in response to dexamethasone. Similar results were found by *Claustrat et al.* (1984) who found that the lower amplitude of the nocturnal secretion of melatonin in depressed patients was associated with hypercortisolemia. In his population, *Wetterberg* (1983) found an association between high serum cortisol, abnormal (DST) results, and low melatonin levels in acute depressive episodes. Even remitted, euthymic patients with normalization of cortisol continued to show low melatonin levels six weeks after recovery. *Nair et al.* (1984) also found reduced nocturnal elevation of melatonin in 6 depressed male patients. Very recently, *Boyce* (1985) reported the absence of a nocturnal increase in 6-sulphatoxy melatonin, a urinary metabolite of mela-

tonin, in three of eight melancholic patients. However, lack of a control group complicates interpretation of these results.

As summarized then, other groups have reported altered concentrations of melatonin in serum in major depression. Our study is the first demonstration of an association between decreased nocturnal melatonin levels and the diagnosis of the melancholic subtype of major depressive disorder. These findings confirm and extend the work of these previous investigators. However, several differences between this work and that of the others are apparent. The reduced values of melatonin in the melancholic patients were confirmed to nocturnal values. Samples obtained in patients at 9 a.m. in New City and samples obtained between 4.30 p.m. and 9 p.m. in patients in Philadelphia showed no differences among groups. This is in contrast to *Wirz-Justice's* and *Arendt's* (1979) observation of lower 8 a.m. values of melatonin in depressives.

Although in this study a decreased nocturnal rise of melatonin was associated with melancholia as was DST non-suppression, a high degree of concordance between abnormal cortisol secretion and diminished nocturnal melatonin secretion was not found. This differs from the findings of others (*Wetterberg*, 1983; *Branchey et al.*, 1982; *Claustrat et al.*, 1984). One possible explanation for the difference is the time of sampling for melatonin. In the patients on whom we did DST's, nocturnal values of melatonin were obtained only at 11 p.m., which is clearly prior to peak nocturnal concentrations (see Fig. 1). In contrast, values were obtained at 2 a.m. in the study by *Wetterberg* (1983) and between 10 p.m. and 7 a.m. in the study of *Claustrat et al.* (1984); such values are more likely to be peak values.

What does seem to be reasonably consistent is the reduced nocturnal rise of melatonin in depressives. Indeed, the similarity of the differences in values between depressed patients and control subjects reported in the studies of *Wetterberg* (1983), *Claustrat et al.* (1984) and our group is striking. The mechanism(s) underlying the lowered nocturnal rise of melatonin in depressives in (are) unknown. Depression may be associated with elevated sympathoadrenal activity (*Koslow et al.*, 1983) which, if prolonged, could cause subsensitivity of pineal beta-adrenergic receptors. Alternatively, low nocturnal levels of melatonin might be an expression of a generalized defect in sensitivity of beta-adrenergic receptors or of sympathetic transmission. Many other explanations are possible.

The sensitivity of this finding of decreased nocturnal secretion of melatonin in depressives is unknown as is its relationship to clinical symptoms such as sleep disturbance, motor activity, or weight loss. The nocturnal rise of melatonin does not seem to be reduced in

schizophrenia (*Wetterberg*, 1982; *Ferrier et al.*, 1982), but whether other diagnostic groups show any amplitude or pattern disturbance of melatonin in serum remains to be seen. Most studies of depressives have focused on group changes the amplitude of melatonin secretion at night. *Claustrat et al.* (1984) additionally sought evidence for rhythm changes but could not show significant phase-advance. *Nair et al.* (1984), though, did report a delay in the nocturnal rise of melatonin together with earlier peak nocturnal values in the depressed patients that they studied. In general what has been ignored might be significant interindividual differences in patterns of secretion. More refined statistical analysis may yield evidence for this. Our preliminary data suggest considerable variability in pattern and time to peak values among patients as compared to the pattern in controls. This might be consistent with a phase-instability on dysregulation hypothesis of depression (*Siever* and *Davis*, 1985).

More sophisticated testing of pineal function may be expected in the future. The technique of *Lewy* and colleagues (1985) of assessing nocturnal sensitivity of melatonin to light suppression is one example. In addition, is should be possible to test the sensitivity of pineal beta-receptors in depressives by lowering peak nocturnal levels of melatonin by the administration of beta-antagonists. Further evidence to confirm or disprove *Wetterberg's* hypothesis (1983) that decreased melatonin secretion is a trait marker of depressive disorder is needed from studies using patients as their own controls over time. If confirmed, such a marker would have potential usefulness in screening asymptomatic relatives of depressed patients.

Desmethylimipramine—induced Effects on the Nocturnal Rise of Melatonin in Depressed Patients

There is no longer any doubt that repeated treatment of rats with antidepressants produces effects on certain monoamine-elicited responses that are very different to those seen after their acute administration. For example, whereas tricyclic antidepressant (TCA)-induced blockade of NE uptake (*Glowinski* and *Axelrod*, (1964) enhances and prolongs certain adrenergic responses measured after their acute administration (*Sigg*, 1959; *Moyer et al.*, 1979; *Heydorn et al.*, 1980), repeated administration of TCAs, monoamine oxidase inhibitors (MAOIs) or electroconvulsive shocks (ECS) to rats reduces responses elicited by activation of beta-adrenergic receptors *in vitro* (*Frazer et al.*, 1974; *Vetulani* and *Sulser*, 1975; see *Olpe*, 1983). The diminished responsiveness is related, at least in part, to the ability of

repeated antidepressant treatments to lower (or "down regulate") the number of beta-adrenergic receptors (*Banerjee et al.,* 1977; see *Olpe,* 1983). The ability of antidepressants to decrease beta-adrenergic receptors depends on the presence of an intact noradrenergic innervation (*Wolfe et al.,* 1978; *Moyer et al.,* 1981) and is presumably a consequence of the persistent overexposure of receptors to transmitter (*Dibner* and *Molinoff,* 1979). Diminished electrophysiological responsiveness of cingulate cortical neurons (*Olpe* and *Schellenberg,* 1980) and cerebellar Purkinje cells (*Schultz et al.,* 1981) to iontophoretically applied NE has been found to occur after repeated treatment of rats with antidepressants. In general, results of the electrophysiological studies agree well with those obtained using ligand binding techniques (see *Olpe,* 1983).

We extended such observations by demonstrating that beta-adrenergic responsiveness was reduced by antidepressants when such responsiveness was measured *in vivo.* In these studies, we used the pineal gland as a model system for measuring beta-adrenergic responsiveness. The pineal gland is a useful model as it contains a high density of beta-receptors (*Greenberg* and *Weiss,* 1978) coupled to a catecholamine sensitive adenylate cyclase capable of generating adenosine 3', 5'-monophosphate (cyclic AMP) (*Weiss* and *Costa,* (1967); the activity of the sympathetic fibers to the pineal is controlled by environmental light, with darkness stimulating their activity (*Brownstein* and *Axelrod,* 1974). Consequently, it is possible to use environmental stimuli to produce activation in the gland.

Both DMI and the MAOI, nialamide, when given to rats repeatedly, but not acutely, reduced the ability of either exogenously administered catecholamine (*Moyer et al.,* 1979; *Moyer et al.,* 1981) or NE released endogenously from sympathetic nerves (see *Weiss et al.,* 1982) to stimulate the content of cyclic AMP in the pineal gland. More recently, we have used this gland to see whether the antidepressant-induced decrease in the ability of NE to raise cyclic AMP has any functional consequences. In other words, would responses mediated by changes in the intracellular concentration of cyclic AMP also be diminished in rats treated repeatedly with antidepressants? In the pineal gland, cyclic AMP increases the rate of synthesis of melatonin by increasing the activity of the enzyme, N-acetyltransferase, that is normally rate-limiting in melatonin synthesis (*Klein et al.,* 1970). Therefore, by measuring melatonin, the net functional effect of antidepressant-induced subsensitivity of the beta-receptor/ adenylate cyclase/cyclic AMP system on end-product formation (*i.e.,* melatonin) can be assessed. It was important to establish that changes in end-product formation would parallel changes in intra-

cellular cyclic AMP produced by antidepressants as we have found
other situations in which this is not so (*Heydorn et al.*, 1983).

We found that the rise in pineal melatonin caused by either
exogenously administered isoproterenol or by darkness was reduced
in rats treated repeatedly with either DMI or nialamide (*Heydorn et
al.*, 1982) (Figs. 3 and 4). Melatonin production by the pineal is
regulated by the beta-adrenergic receptor—adenylate cyclase—cyclic
AMP system (*Wurtman et al.*, 1971); because repeated, but not acute,
administration of antidepressants reduces both the density of pineal
beta-adrenergic receptors and catecholamine—stimulated production
of cyclic AMP (*Moyer et al.*, 1981; *Heydorn et al.*, 1982), it is likely that
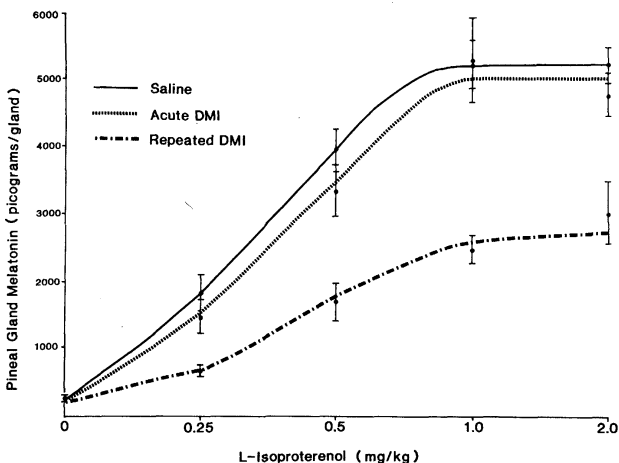

Fig. 3. Effect of acute or repeated treatment of rats with DMI on the L-isoproterenol-
induced elevation of melatonin concentration in the pineal gland. Rats received
either an acute injection of DMI (10 mg/kg) or the same dose twice daily for 7 days.
Animals treated acutely were killed 5 hours later, whereas those treated repeatedly
were killed 24 hours after the final injection. Three hours before decapitation, L-iso-
proterenol was administered s.c. Control rats received 0.9% NaCl. The pineal gland
content of melatonin was determined by radioimmunoassay. Results are shown as the
mean ± S.E.M. for 7 to 14 rats. Acute treatment of rats with DMI did not alter the
ability of L-isoproterenol to raise melatonin concentrations. The L-isoproterenol-
induced elevation of melatonin was reduced significantly in rats given DMI
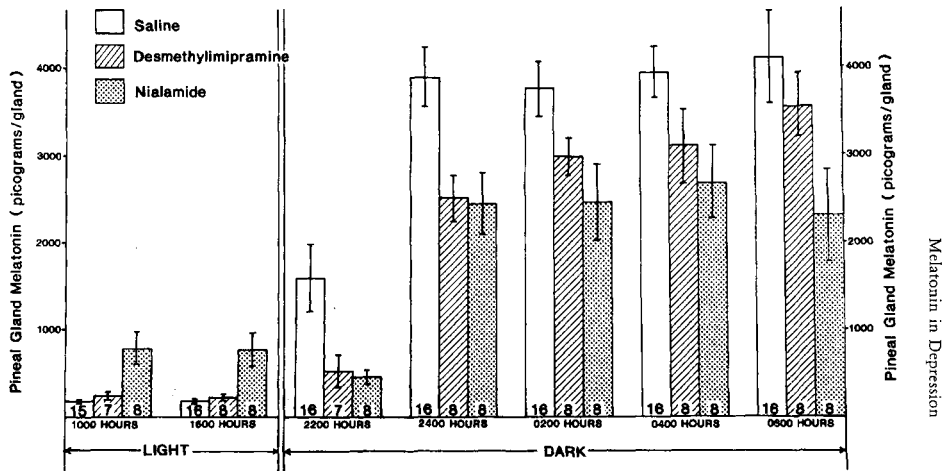repeatedly ($p < 0.01$) (from *Heydorn et al.*, 1982)

Fig. 4. Effect of repeated treatment of rats with DMI or nialamide on the concentration of melatonin in pineal gland at different times during the day. Animals were given either DMI (10 mg/kg, twice daily), nialamide (40 mg/kg, twice daily) or 0.9% NaCl for 7 days. They were killed 24 hours after the final injection at different times throughout the day. Each bar and bracket represents the mean value of melatonin ± S.E.M. The number of experiments is shown within each bar. In control rats, the concentration of melatonin in the pineal gland was significantly higher at each nighttime measurement than that measured at either daytime point ($p < 0.005$). Compared to saline-treated animals, treatment of rats with nialamide significantly raised the concentrations of melatonin measured at 10 a.m. and 4 p.m. ($p < 0.01$), whereas treatment with DMI had no effect at these times. The nighttime rise of melatonin was reduced significantly in rats treated with either DMI ($p < 0.05$) or nialamide ($p < 0.01$) as compared with that in rats receiving only saline and killed at night (from *Heydorn et al.*, 1982)

the diminished hormonal response of the pineal to catecholamines is due to antidepressant—induced subsensitivity of beta-adrenergic receptors.

An advantage of using darkness to stimulate melatonin in the pineal is that it allows an overall assessment of the effect of drug treatments on the final output of a multisynaptic pathway. Since the antidepressants lowered the nocturnal rise of melatonin, it seems reasonable to conclude that the net effect of these drugs on responses elicited by NE activating beta-adrenergic receptors in inhibitory.

In large measure, these results have been confirmed (*Cowen et al.,* 1983 a; *Friedman et al.,* 1984). However, *Cowen et al.* (1983 a) did not find DMI treatment to reduce the darkness-induced rise of melatonin although such treatment did reduce the rise caused by iso-proterenol. There are several important differences between their study and ours. Perhaps the most important is that they gave the rats half the dose of DMI (10 mg/Kg, once daily) that we did (10 mg/Kg, bid). This would obviously produce a lower mean daily plasma concentration of DMI in their study than in ours and could account for their finding a much smaller reduction ($< 20\%$) in pineal beta-receptors than we did (about 40%). Decreases in beta-receptors greater than 20% may be necessary in order to demonstrate significant inhibition of the darkness-induced rise in melatonin. Alterations in the darkness-induced rise of melatonin may also be more difficult to detect than changes in the rise due to isoproterenol as in our study DMI reduced the rise of melatonin caused by isoproterenol to a much greater extent than the rise due to darkness (about 50% inhibition versus about 25% inhibition).

The results of a clinical study we did however, cause us to believe that the results obtained by *Cowen et al.* (1983 a) may be more applicable to patients who are treated with DMI. Because we found DMI-treatment to produce effects on the night-time rise of serum melatonin similar to its effects on melatonin in the pineal (*Heydorn et al.,* 1982), we wanted to see if a similar result would be obtained in patients treated with this drug. As mentioned previously, the similarity of the mechanisms responsible for the nocturnal rise of melatonin in both rats and humans provides the rationale for such a study. Based on results of our animal experiments, we expected to find treatment of depressed patients with DMI reducing the night-time rise of melatonin in their serum.

Seven male depressed patients (36—60 years) judged sufficiently depressed (Hamilton Score, 30 ± 5) to require hospitalization and a trial with an antidepressant, entered the study. On the day before treatment with DMI was initiated and again after 4 weeks of treat-

ment, blood was taken from an indwelling venous catheter every 1.5 hours between 4.30 p.m. and 7.30 a.m. Lights were turned off at 10 p.m. and turned on at 6 a.m. During the dark phase, the room was completely darkened and blood was drawn with the aid of a dim red light. Melatonin in serum was measured by radioimmunoassay (*Rollag* and *Niswender*, 1976).

The results of the study are shown in figures 5 and 6. The data were analyzed by a two-way ANOVA for repeated measures. Both before and during treatment with DMI, serum melatonin rose significantly during the dark phase ($F = 7.4$; $p < 0.001$). Prior to treatment,
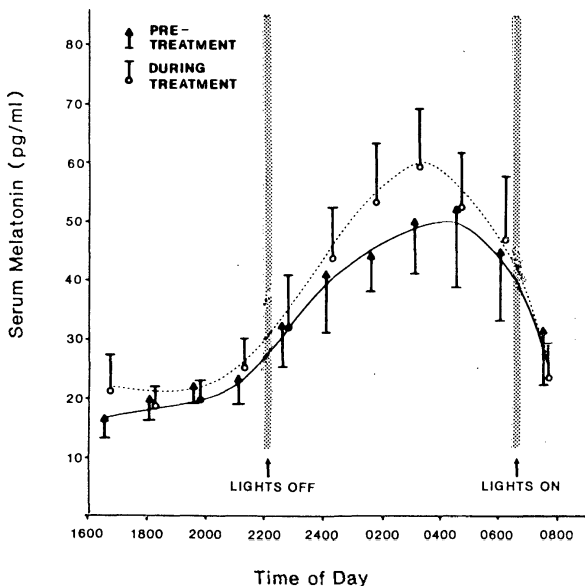

Fig. 5. The concentration of melatonin in serum of seven depressed patients before and after four weeks of treatment with DMI. Each point represents the mean ± S.E.M. Melatonin concentrations rose significantly during the dark phase. However, treatment of patients with DMI had no significant effect on the concentration of melatonin in serum measured over time. The mean (± S.E.M.) concentration of DMI in plasma of the patients was 93 ±17 ng/ml
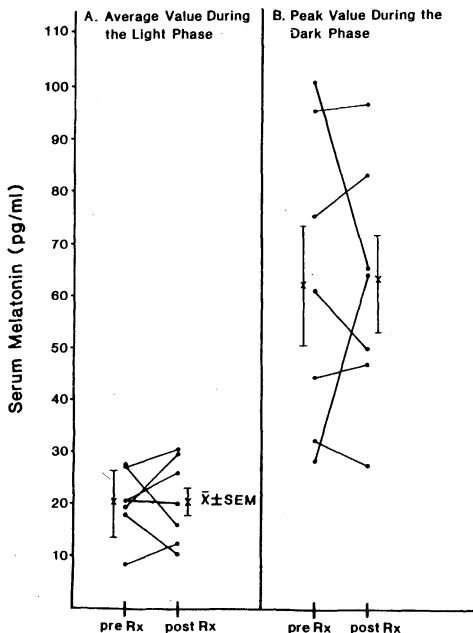
Fig. 6. The concentration of melatonin in serum of seven depressed patients before and after four weeks of treatment with DMI. Range shown is mean ± S.E.M. Treatment of patients with DMI had no significant effect on either the mean value of melatonin in serum measured before the lights were turned off or the peak nocturnal value of melatonin

the average value of serum melatonin measured before the lights were turned off was 20 ± 6.5 pg/ml and this rose to a peak value during the dark phase of 62 ± 11 pg/ml. Treatment of the patients with DMI had no significant effect on concentrations of melatonin in serum ($F = 0.39$, NS); during treatment with DMI, the average concentration of melatonin in serum before the lights were turned off was 21 ± 3 pg/ml and rose to a peak value of 62 ± 8.8 pg/ml.

Thus, the results obtained in depressed patients treated with DMI are different from that found in rats given the tricyclic anti-

depressant. A likely explanation for the difference in results between rats and humans is the lower concentration of DMI in the plasma of the patients (93 ± 17 ng/ml, measured 7–12 hours after the last dose) as compared to that in the rats (347 ± 75 ng/ml, measured 24 hours after the final dose). Under usual clinical use, plasma concentrations of DMI may not be sufficiently high to cause the degree of down-regulation of pineal beta-adrenergic receptors necessary to produce a reduction in the darkness-induced rise of melatonin. Alternatively, it may be that acute treatment of patients with DMI would enhance the nocturnal rise of melatonin. We did not evaluate this. Were this to occur, though, then the development of beta-receptor down-regulation over a longer period of treatment would be expected to return the hypothesized elevated rise of melatonin at night toward pre-treatment values.

It is unfortunate that in humans exogenously administered catecholamines do not raise serum melatonin (*Vaughan et al.*, 1976; *Moore et al.*, 1979). Both our results and those of *Cowen et al.* (1983 a) make it evident that in the rat the l-isoproterenol-induced rise of melatonin provides a more sensitive measure of the DMI-induced decrease in beta-receptors than the rise caused by darkness.

There has been very little other work on the effect of antidepressant treatments on levels of melatonin in serum. Although we found no effect of treatment with DMI, *Thompson et al.* (1983) found such treatment to cause a significant enhancement of the nocturnal rise of melatonin in three depressives. This was observed after either one or three weeks of treatment. Consistent with this observation is the brief report of *Golden et al.* (1985) of increased urinary excretion of 6-hydroxymelatonin in five patients upon treatment with DMI. Reasons for differences between these reports and our observations are unknown. Clearly, the total number of patients studied remains small. More widespread use of the improved analytical methodology for measuring serum melatonin or its urinary metabolites should enable the true picture of the effect of antidepressants on melatonin secretion to emerge.

In summary, measurement of melatonin provides an opportunity to evaluate adrenergic responsiveness in man. Results obtained to date on the nocturnal secretion of melatonin are reasonably consistent in demonstrating a reduced nocturnal rise of this hormone from the pineal gland. Whether this is a reflection of a generalized alteration in adrenergic responsiveness in depressives remains to be established. Similarly, more information needs to be provided to determine if this is a primary alteration or rather secondary to some other change (*e.g.*, anxiety, insomnia, increased HYPAC activity) in

depression. Finally, although we did not find antidepressant-treat-
ment to change the nocturnal rise of melatonin [indicating perhaps,
that this is a trait-marker of the illness as suggested previously by *Wet-
terberg* (1983)], much more work will be needed to resolve this
important issue.

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References

Arendt J, Wetterberg L, Heyden T, Sizonenko PC, Paunier L (1977) Radio-
    immunoassay of melatonin: Human serum and cerebrospinal fluid.
    Human Res 8: 65–75
Axelrod J (1978) Introductory remarks on regulation of pineal indoleamine
    synthesis. J Neural Transm [Suppl] 13: 73–79
Banerjee SP, Kung LS, Riggi SJ, Chanda SK (1977) Development of Beta-
    adrenergic receptor subsensitivity by antidepressants. Nature (London)
    268: 455–456
Beckman H, Goodwin FK (1980) Urinary MHPG in subgroups of depressed
    patients and normal controls. Neuropsychobiology 6: 91–100
Bowden CL, Koslow SH, Hanin I, Mass JW, Davis JM, Robins E (1985)
    Effects of amitriptyline and imipramine on brain amine neurotransmit-
    ter metabolites in cerebrospinal fluid. Clin Pharmacol Therap 37: 316–
    324
Boyce PM (1985) 6-Sulphatoxy melatonin in melancholia. Am J Psychiatry
    142: 125–127
Branchey L, Weinberg U, Branchey M, Linkowski P, Mendlewicz J (1982)
    Simultaneous study of 24-hour patterns of melatonin and cortisol secre-
    tion in depressed patients. Neuropsychobiology 8: 225–232
Brown R, Kocsis JH, Caroff S, Amsterdam J, Winokur A, Stokes PE, Frazer A
    (1985) Differences in nocturnal melatonin secretion between
    melancholic depressed patients and control subjects. Am J Psychiatry
    142: 811–816
Brownstein M, Axelrod J (1974) Pineal gland: 24-hour rhythm in
    norepinephrine turnover. Science 184: 163–165
Charney DS, Menkes DB, Heninger GR (1981) Receptor sensitivity and the
    mechanism of action of antidepressant treatment: Implications of the
    etiology and therapy of depression. Arch Gen Psychiatry 38: 1160–1180

Cowen PJ, Fraser S, Grahame-Smith DG, Green AR, Stanford C (1983 a) The effect of chronic antidepressant administration on Beta-adrenoceptor function of the rat pineal. Br J Pharmacol 78: 89–96

Cowen PJ, Fraser S, Sammons R, Green AR (1983 b) Atenolol reduces plasma melatonin concentration in man. Br J Clin Pharmacol 15: 579

Claustrat B, Chazot G, Brun J, Jordan D, Sassolas G (1984) A chronobiological study of melatonin and cortisol secretion in depressed subjects: Plasma melatonin, a biochemical marker in major depression. Biol Psychiatry 19: 1215–1217

Dibner MD, Molinoff PB (1979) Agonist-induced changes in beta-adrenergic receptor density and receptor–mediated responsiveness in slices of rat cerebral cortex. J Pharmacol Exp Therap 210: 433–439

Frazer A, Pandey G, Mendels J, Neeley S, Kane M, Hess ME (1974) The effect of triiodothyronine in combination with imipramine on (^{3}H)-cyclic AMP production in slices of rat cerebral cortex. Neuropharmacology 13: 1131–1140

Friedman E, Yocca FD, Cooper TB (1983) Antidepressant drugs with varying pharmacological profiles alter rat pineal beta adrenergic-mediated function. J Pharmacol Exp Therap 228: 545–550

Glowinski J, Axelrod J (1964) Inhibition of uptake of tritiated-noradrenaline in the intact rat brain by imipramine and structurally related compounds. Nature (London) 204: 1318–1319

Golden RN, Markey SP, Potter WZ (1985) A new marker for noradrenergic function in man. Am Psychiatry Assoc (New Res Abstr) NR 51

Greenberg L, Weiss B (1978) Beta-adrenergic receptors in aged rat brain: Reduced number and capacity of pineal gland to develop supersensitivity. Science 201: 61–63

Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23: 56–62

Hanssen T, Heyden T, Sundberg I, Wetterberg L (1977) Effect of propranolol on serum-melatonin (letter). Lancet ii: 309–310

Heydorn WE, Brunswick DJ, Frazer A (1982) Effect of treatment of rats with antidepressants on melatonin concentrations in the pineal gland and serum. J Pharmacol Exp Therap 222: 534–543

Heydorn WE, Lucki I, Frazer A (1983) Continous light paradoxically reduces catecholamine-induced melatonin production. Brain Res 267: 175–178

Heydorn WE, Mendels J, Frazer A (1980) Effects of darkness and of desmethylimipramine on pineal gland concentrations of adenosine 3', 5'-monophosphate. Biochem Pharmacol 29: 1341–1346

Jimerson DC, Lynch HJ, Post RM, Wurtman RJ, Bunney WE (1977) Urinary melatonin rhythms during sleep deprivation in depressed patients and normals. Life Sci 20: 1501–1508

Jones RL, McGeer PL, Greiner AC (1969) Metabolism of exogenous melatonin in schizophrenic and non-schizophrenic volunteers. Clin Chim Acta 26: 281–285

Kennaway DJ, Frith RG, Phillipou G, Matthews CD, Seamark RF (1977) A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography/mass spectrometry. Endocrinology 101: 119—127

Klein DC, Berg GR, Weller J (1970) Melatonin synthesis: Adenosine 3′, 5′-monophosphate and norepinephrine stimulate N-acetyltransferase. Science 168: 979—980

Kneisley LW, Moskowitz MA, Lynch HJ (1978) Cervical spinal cord lesions disrupt the rhythm in human melatonin excretion. J Neural Transm [Suppl] 13: 311—323

Kopin IJ, Pare CMB, Axelrod J, Weissbach H (1961) The fate of melatonin in animals. J Biol Chem 236: 3072—3075

Lewy AJ (1981) Human plasma melatonin studies: Effects of light and implications for biological rhythm research. In: Birau N, Schloot W (eds) Melatonin—current status and perspectives: advances in the biosciences, vol 29. Pergamon Press, New York, pp 397—400

Lewy AJ, Nurnberger JI, Wehr TA, Pack D, Becker LE, Powell R-L, Neusome DA (1985) Supersensitivity to light: Possible trait marker for manic-depressive illness. Am J Psychiatry 142: 725—727

Lewy AJ, Wehr RA, Gold PW, Goodwin FK (1979) Plasma melatonin in manic-depressive illness. In: Usdin E, Kopin IJ, Barchas J (eds) Catecholamines: basic and clinical frontiers. Pergamon Press, New York, pp 1173—1175

Linnoila M, Karoum F, Calil HM, Kopin IJ, Potter WZ (1982) Alteration of norepinephrine metabolism with desipramine and zimelidine in depressed patients. Arch Gen Psychiatry 39: 1025—1028

Mass JW, Fawcett J, Dekirmenjian H (1968) 3-Methoxy-4-hydroxyphenyl-glycol (MHPG) excretion in depressive states: A pilot study. Arch Gen Psychiatry 19: 129—134

Mendlewicz J, Linkowski P, Branchey L, Weinberg V, Weitzman ED, Branchey M (1979) Abnormal 24-hour pattern of melatonin secretion in depression (letter). Lancet ii: 1362

Moore DC, Paunier L, Sizonenko PC (1979) Effect of adrenergic stimulation and blockade on melatonin secretion in the human. Prog Brain Res 52: 517—521

Moore RY (1978) Neural control of pineal function in mammals and birds. J Neural Transm [Suppl] 13: 47—58

Moyer JA, Greenberg LH, Frazer A, Brunswick D, Mendels J, Weiss B (1979) Opposite effects of acute and repeated administration of desmethylimipramine on adrenergic responsiveness in rat pineal gland. Life Sci 24: 2237—2244

Moyer JA, Greenberg LH, Frazer A, Weiss B (1981) Subsensitivity of the beta-adrenergic receptor-linked adenylate cyclase system of rat pineal gland following repeated treatment with desmethylimipramine and nialamide. Mol Pharmacol 19: 187—193

Nair NPV, Hariharasubramanian N, Pilapil C (1984) Circadian rhythm of plasma melatonin in endogenous depression. Prog Neuropsychopharmacol Biol Psychiatry 8: 715—718

Olpe H-R (1983) Depression: Molecular and psychological based therapies. Korf J, Pepplinkhuizen L (eds) TGO Foundation, pp 21–45

Olpe H-R, Schellenberg A (1980) Reduced sensitivity of neurons to noradrenergic stimulation after chronic treatment with antidepressant drugs. Eur J Pharmacol 63: 7–13

Prange AJ (1964) The pharmacology and biochemistry of depression. Dis Nerv System 25: 217–221

Rollag MD, Niswender GD (1976) Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinology 98: 482–489

Ross RJ, Scheinin M, Lesieur P, Rudorfer MV, Hauger RL, Siever LJ, Linnoila M, Potter WZ (1985) The effect of clorgyline on noradrenergic function. Psychopharmacology 85: 227–230

Schildkraut JJ (1965) The catecholamine hypothesis of affective disorders: A review of supporting evidence. Am J Psychiatry 122: 509–522

Schultz JE, Siggins GR, Schocker FW, Turck M, Bloom FE (1981) Effects of prolonged treatment with lithium and tricyclic antidepressants on discharge frequency, norepinephrine responses and beta-receptor binding in rat cerebellum: Electrophysiological and biochemical comparison. J Pharmacol Exp Therap 216: 28–38

Seiver LJ, Davis KL (1985) Overview: Toward a dysregulation hypothesis of depression. Am J Psychiatry 142: 1017–1031

Sigg EB (1959) Pharmacological studies with tofranil. Canad Psychiat Assoc J 4 [Suppl]: 75–85

Sisak ME, Markey SP, Colburn RW, Zavadil AP III, Kopin IJ (1979) Identification of 6-hydroxymelatonin in normal human urine by gas chromatography/mass spectrometry. Life Sci 25: 803–806

Stokes PE, Stoll PM, Koslow SH, Maas JW, Davis JM, Swann AC, Robins E (1984) Pretreatment DST and hypothalamic-pituitary-adrenocortical function in depressed patients and comparison groups. Arch Gen Psychiatry 41: 257–267

Thompson C, Checkley SA, Corn T, Franey C, Arendt J (1983) Down-regulation at pineal Beta-adrenoceptors in depressed patients treated with desipramine? Lancet i: 1101

Vaughan GM, McDonald SD, Jordan RM, Allen JP, Bell R, Stevens EA (1979) Melatonin, pituitary function and stress in humans. Psychoneuroendocrinology 4: 351–362

Vaughan GM, Pelham RW, Pang SF, Loughlin LL, Wilson KM, Sandock KL, Vaughan MK, Koslow SH, Reiter RJ (1976) Nocturnal elevation of plasma melatonin and urinary 5-hydroxyindoleacetic acid in young men: Attempts at modification by brief changes in environmental lighting and sleep and by autonomic drugs. J Clin Endocrinol Metab 42: 752–764

Veith RC, Raskine MA, Barnes RF, Gumbrecht G, Ritchie JL, Halter JB (1983) Tricyclic antidepressants and supine standing and exercise plasma norepinephrine levels. Clin Pharmacol Therap 33: 763–769

Vetulani J, Sulser F (1975) Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. Nature (London) 257: 495–496

Weiss B, Costa E (1967) Adenyl cyclase activity in rat pineal gland: Effects of chronic denervation and norepinephrine. Science 156: 1750–1752

Weiss B, Heydorn W, Frazer A (1982) Modulation of beta-adrenergic receptor-adenylate cyclase system following acute and repeated treatment of rats with antidepressants. In: Costa E, Racagni G (eds) Typical and atypical antidepressants: molecular mechanisms. Raven Press, New York, pp 37–53

Wetterberg L (1983) The relationship between the pineal gland and the pituitary-adrenal axis in health, endocrine, and psychiatric conditions. Psychoneuroendocrinol 8: 75–80

Wetterberg L (1978) Melatonin in humans: physiological and clinical studies. J Neurol Transm [Suppl] 13: 289–310

Wetterberg L, Beck-Friis J, Aperia B, Petterson V (1979) Melatonin/cortisol ratio in depression (letter). Lancet ii: 1361

Wolfe BB, Harden TK, Sporn JR, Molinoff PB (1978) Presynaptic modulation of beta-adrenergic receptors in rat cerebral cortex after treatment with antidepressants. J Pharmacol Exp Therap 207: 446–457

Wurtman RJ, Shein HM, Larin F (1971) Mediation by beta-adrenergic receptors of effects of norepinephrine on pineal synthesis of [^{14}C]-serotonin and [^{14}C]-melatonin. J Neurochem 18: 1683–1689

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