Psychiatry Research, 32:183-189
Elsevier

Pineal-Adrenal Interactions: The Effect of Acute Pharmacological Blockade of Nocturnal Melatonin Secretion

Mark A. Demitrack, Alfred J. Lewy, and Victor I. Reus

Received August 2, 1989; revised version received January 26, 1990; accepted March 20, 1990.

Abstract. The pineal gland is a complex neuroendocrine organ which is under photoneuroendocrine control. Previous studies in animals and humans have suggested reciprocal variations in pineal melatonin biosynthesis and adrenal glucocorticoid output; it remains unclear, however, whether a causal relationship exists between these two systems. To address this question, we examined the overnight secretory activity of the hypothalamic-pituitary-adrenal (HPA) axis in conjunction with acute pharmacological suppression of pineal melatonin biosynthesis in 11 healthy volunteers. Results of the current study are consistent with the hypothesis that melatonin does not function as a tonic inhibitor of the HPA axis on an acute basis.

Key Words. Pineal gland, 6-hydroxymelatonin, cortisol, β-adrenergic receptor.

The pineal gland is a complex neuroendocrine organ which sits at the interface of the blood-brain barrier, where it can release its products into the cerebrospinal fluid and the systemic circulation (Pevet, 1982). It is under photoneuroendocrine control, producing its major biosynthetic product, *N*-acetyl-5-methoxytryptamine or melatonin, during darkness (Lewy, 1982).

Norepinephrine is the principal regulatory signal for melatonin biosynthesis, stimulating both β_1- and α_1-adrenergic receptors on the pinealocyte (Klein et al., 1983). This leads to activation of the cyclic adenosine monophosphate and inositol phosphate second messenger pathways, with synthesis of the enzyme serotonin-*N*-acetyltransferase (SNAT) and the production of melatonin (Klein et al., 1983; Sugden et al., 1985). In addition to norepinephrine, a number of neuropeptide systems are known to impinge on the pineal gland either through peptidergic projections (e.g., vasopressin and oxytocin) or via receptors located on the pinealocyte surface (e.g., vasoactive intestinal peptide, substance P) (Ebadi, 1987). The functional effect of these multiple neurochemical inputs on the noradrenergic regulation of pineal activity is unknown.

A variety of animal studies have suggested a relationship between the pineal gland and the hypothalamic-pituitary-adrenal (HPA) axis (Wurtman et al., 1959, 1961;

Mark A. Demitrack, M.D., is Assistant Professor and Director of Clinical Research, Michigan Eating Disorders Program, Department of Psychiatry, University of Michigan, Ann Arbor, MI. Alfred J. Lewy, M.D., Ph.D., is Professor of Psychiatry, Ophthalmology, and Pharmacology and Director of the Sleep and Mood Disorders Laboratory and the Mass Spectrometry Laboratory, The Oregon Health Sciences University, Portland, OR. Victor I. Reus, M.D., is Professor and Medical Director, Langley Porter Hospital, University of California, San Francisco, CA. (Reprint requests to Dr. M.A. Demitrack, Michigan Eating Disorders Program, 8D8806/UH 0116, University of Michigan, 1500 E. Medical Center Dr., Ann Arbor, MI 48109-0116, USA.)

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Vaughan et al., 1972). Studies in humans have suggested the possibility of an inverse association between pineal and adrenal activity in affective illness (Beck-Friis et al., 1985; Frazer et al., 1986); it is unclear, however, whether there is a causal relationship in either direction.

To examine further the possible association between activity of the pineal gland and the HPA axis in normal individuals, we examined the overnight secretory activity of the HPA axis in conjunction with acute pharmacological suppression of nocturnal melatonin release. Blockade of nocturnal melatonin release was obtained with the β-adrenergic antagonists propranolol and atenolol. We chose to compare the effects of these two agents since propranolol is a nonselective, lipophilic β-adrenergic receptor antagonist and therefore blocks both β_1- and β_2-adrenergic receptors centrally and peripherally. Atenolol, on the other hand, is a selective β_1-adrenergic receptor, nonlipophilic β-blocker. Therefore, it may be theoretically more selective in blocking the peripherally located β_1-adrenergic receptors on the pinealocytes while having little penetration into the central nervous system. Parenthetically, catecholaminergic stimulation of pituitary release of adrenocorticotropic hormone (ACTH) appears to occur via β_2-adrenergic receptors, suggesting little effect of atenolol on this mechanism (Mezey et al., 1983).

Methods

Subjects. Eleven subjects (7 males, 4 females, age range 20-33) were recruited. All subjects were interviewed before the study and underwent a complete physical examination to rule out significant medical or psychiatric illness. All subjects refrained from using medications, alcohol, and caffeine and had been on a regular schedule of 8-9 hours of sleep (10:30-11:30 p.m. until 6:30-7:30 a.m.) for 2 weeks before each study night.

Procedure. Three separate studies were performed, at least 2 weeks apart from one another. All subjects underwent studies in the same order. For study 1, subjects were instructed to collect all urine voided after 6 p.m., up to and including a final void at 10 a.m. the following morning. Study 2 involved a urine collection as above. In addition, at 6 p.m. during study 2, each subject was given a dose of 60 mg of propranolol. Pulse and blood pressure were then monitored for the next 2 hours. At 10:30 p.m., an additional dose of 40 mg of propranolol was given. Pulse and blood pressure were again monitored for an hour. Study 3 was similar in format to study 2 with the substitution of atenolol for propranolol in dosages of 50 mg and 25 mg at 6 p.m. and 10:30 p.m., respectively. No patients reported adverse effects from the dosages of β-blockers administered.

Urine was aliquoted within 2 hours of final collection and frozen at -70 °C until assayed. Urinary free cortisol was measured by competitive protein binding assay as previously described (Contreras et al., 1986). Intra-assay and interassay variabilities were 7.6% and 12.5%, respectively. The principal urinary metabolite of melatonin, 6-hydroxymelatonin, was assayed by gas chromatography/mass spectrometry, as previously described (Tetsuo et al., 1981). Intra-assay and interassay variabilities were $< 6\%$.

Results

Acute administration of either 100 mg of propranolol or 75 mg of atenolol resulted in an almost complete pharmacological ablation of the nocturnal surge of 6-hydroxymelatonin excretion (baseline mean ± SD = 13721 ± 4945 ng/TV vs. 1413 ± 1138 ng/TV on propranolol, $p < 0.006$, and 2961 ± 1850 ng/TV on atenolol, $p < 0.006$) (Fig. 1*a*). However, no significant differences were seen in overnight urinary free cortisol

excretion between study 1 and either of the two subsequent studies (baseline mean ± SD = 31 ± 4 μg/TV vs. 35 ± 7 μg/TV on propranolol and 30 ± 3 μg/TV on atenolol) (Fig. 1*b*).

Significant reductions in pulse and blood pressure were noted during the treatment nights (Table 1). No discomfort was reported by any subject.

Fig. 1*a*. Effect of acute beta-adrenergic receptor blockade on overnight secretion of 6-hydroxymelatonin

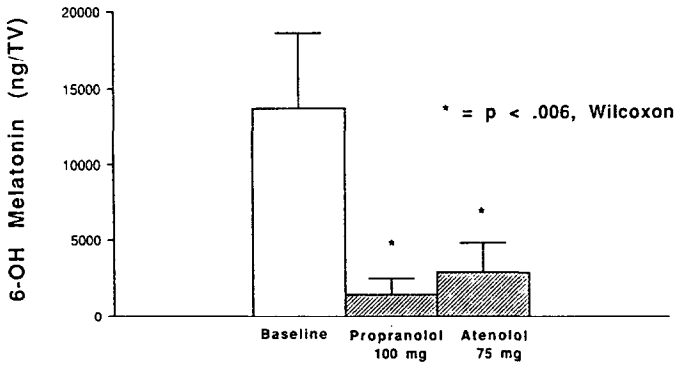

Fig. 1*b*. Effect of acute beta-adrenergic receptor blockade on overnight secretion of cortisol

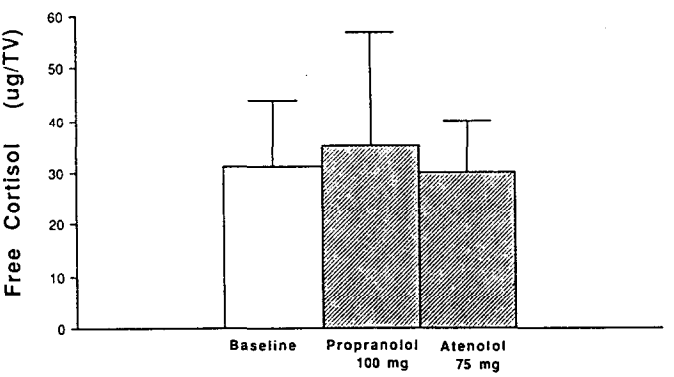

Table 1. Effect of beta blockade on heart rate and blood pressure (BP)

	Baseline	Propranolol	Baseline	Atenolol
Pulse	76 ± 7	56 ± 3[1]	72 ± 7	55 ± 6[1]
Systolic BP	114 ± 7	100 ± 6[1]	116 ± 10	99 ± 6[1]
Diastolic BP	79 ± 7	69 ± 6[1]	75 ± 7	65 ± 6[1]

Note. Data are presented as mean ± SD.

1. $p < 0.01$, paired t test.

Discussion

In the present study, we have shown that acute pharmacological blockade of melatonin biosynthesis was not associated with a concomitant rise of overnight cortisol secretion. In addition, we have replicated previous findings that both propranolol and the nonlipophilic, β_1-selective adrenergic receptor antagonist atenolol produce a dramatic reduction of the nocturnal melatonin surge (Cowen et al., 1983; Arendt et al., 1985). The absence of an inverse association between melatonin and cortisol after reduction in melatonin secretion is consistent with the hypothesis that melatonin does not function as a tonic inhibitor of the HPA axis on an acute basis.

A variety of studies in animals have suggested an inhibitory effect of melatonin on cortisol secretion (Wurtman et al., 1959, 1961; Vaughan et al., 1972). Although the mechanism of melatonin-induced decrease of adrenal gland weight was not definitively elucidated, data indicating a concomitant decrease in plasma ACTH secretion strongly suggest a hypothalamic or pituitary mechanism may be involved (Kinson et al., 1968).

Investigation of the role of glucocorticoids on pineal biosynthesis has been less extensively reported. The definitive presence of glucocorticoid receptors in pinealocytes has not been noted, although ^{3}H-dexamethasone concentrates in the pineal gland (Warembourg, 1975). In cell culture, glucocorticoids have been reported to inhibit the melatonin increase seen with noradrenergic stimulation (Fevre-Montagne and Abou-Samra, 1983). Lipocortin, the tissue mediator of glucocorticoid effects on prostaglandin biosynthesis, is widely distributed throughout the body, including the brain (Wallner et al., 1986). In this regard, it is interesting that in whole organ pineal cultures, glucocorticoids appear to inhibit the noradrenergically stimulated release of arachidonic acid, possibly through lipocortin-dependent inhibition of phospholipase A2 activity (Demitrack et al., unpublished observations). A possible developmental role for glucocorticoids in the modulation of pineal biosynthetic activity has been suggested by the work of Yuwiler (1985), who demonstrated that neonatal exposure to glucocorticoids in the rat produces a reduction of noradrenergically stimulated SNAT activity in the adult rat.

Clinical studies in humans suggest a complex relationship between the pineal and adrenal glands. In studies of affectively ill individuals, a number of investigators have suggested an inverse relationship between melatonin and cortisol, with a blunting of the normal nocturnal melatonin rise (Beck-Friis et al., 1983; Frazer et al., 1986), although this finding has not been universally replicated (Thompson et al., 1988). In some instances, this abnormality appears to persist into the euthymic state (Beck-Friis et al., 1983), a finding which may be of interest in light of the work of Yuwiler noted above (i.e., peristent reduction of nocturnal melatonin may occur as a consequence of repeated activation of the HPA axis in early life. Interestingly, acute administration of glucocorticoids to normal subjects has not been reliably shown to produce a reduction in melatonin secretion at night (Beck-Friis et al., 1983; Demisch et al., 1987).

Studies in the other clinical populations with manifest hypercortisolism also suggest that the reduction in nocturnal melatonin secretion does not simply occur as a consequence of glucocorticoid restraint of pinealocyte function. For example, reports of patients with anorexia nervosa (Dalery et al., 1985; Brambilla et al., 1988) or Cushing's syndrome (Young, 1981) demonstrate normal or elevated amplitude of

melatonin rhythm. However, in a study of 10 patients with ACTH-producing adenomas, Werner et al. (1980) reported that the mean nighttime peak of melatonin secretion was significantly reduced. Parenthetically, Kennedy et al. (1989) have demonstrated a blunting of the nocturnal melatonin surge in a population of patients with anorexia nervosa or bulimia nervosa who were also significantly depressed, but not in a nondepressed group of patients with eating disorders. This suggests that abnormalities of the nocturnal melatonin rhythm again may not occur as a simple consequence of hypercortisolism alone, but may reflect defects in pineal function that are intrinsic to the pathophysiology of the depressed state.

As mentioned above, a variety of neuropeptide systems impinge on the pineal gland, either through peptidergic projections from other brain locations (Buijs and Pevet, 1980) or via receptors present on the pinealocyte membrane (Ebadi, 1987). As the neuropeptide milieu of the cerebrospinal fluid varies significantly in the illness states mentioned, such variations may play a role in modulating the pineal response to hypercortisolism.

The findings of this pilot study demonstrate that acute reductions in melatonin levels induced by β-blockers are not associated with concomitant increases in cortisol secretion. This is consistent with the hypothesis that in the normal individual melatonin does not serve as a tonic inhibitor of cortisol release. It should be noted that the dose of propranolol chosen in this study resulted in a mean reduction of melatonin from baseline of 90% compared with a mean reduction of 80% with the administration of atenolol. While these results are comparable, the possibility cannot be excluded that a higher dose of atenolol would have yielded positive results that were otherwise obscured by small but significant levels of circulating melatonin. As a corollary finding, it is also of interest that acute challenge with propranolol did not appear to affect cortisol secretion. As noted previously, catecholamine modulation of HPA axis function may occur via direct pituitary stimulation of β_2-adrenergic receptors. The results obtained in the present study would suggest that catecholamines do not tonically stimulate HPA axis activity on an acute basis.

In sum, it remains to be determined whether, in the normal individual, more chronic alterations of adrenal activity or biosynthesis of melatonin may be associated with one another. Further, associated disturbances of these two systems in pathological states such as depression and anorexia nervosa may differ significantly from the normal individual and should be interpreted in light of known alterations in other neuroendocrine axes.

Acknowledgments. The authors thank Dr. Sanford Markey of the National Institute of Mental Health for graciously supplying the deuterated 6-hydroxymelatonin internal standard. The project was supported in part by BRSG Grant S07-RR05755 (M.A.D.) awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

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