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Human Melatonin Production Decreases With Age

Robert L. Sack, Alfred J. Lewy, Danielle L. Erb, William M. Vollmer, and Clifford M. Singer

Sleep and Mood Disorders Laboratory, Department of Psychiatry, Oregon Health Sciences University, Portland

The purpose of this study was to investigate the effects of time of year and demographic variables on the amplitude of melatonin production in normal human subjects. Melatonin production was estimated by measuring the overnight excretion of its major urinary metabolite, 6-hydroxymelatonin. Urine was collected on three consecutive nights in the summer from a sample of 60 normal subjects balanced for sex and age. The collections were repeated in a subgroup during the winter. Melatonin production clearly declined with age but was not influenced by other demographic variables or by season of the year.

Key words: 6-hydroxymelatonin, pineal gland, seasonal rhythms, demographic variables, aging

Melatonin (aMT) is produced by the pineal gland mainly at night during the dark. The timing of its production is regulated by an endogenous clock located in the suprachiasmatic nucleus of the hypothalamus that is entrained to the light-dark cycle by visual input from the retinohypothalamic tract [reviewed by Reiter, 1980; Lewy, 1983; Vaughan, 1984]. Although in some species aMT is an important regulator of seasonal rhythms such as breeding and migration [Reiter, 1980], its function in humans remains uncertain.

We undertook this study in order to investigate the effects of time of year and demographic variables on the amplitude of aMT production in normal human subjects. Previous animal studies have shown that the duration of nighttime aMT production decreases during the short scotoperiods (dark periods) of summer [Rollag et al., 1978; Kennaway et al., 1983; Illnerova and Vanecek, 1980] and that aMT production declines with age [Reiter et al., 1980a,b, 1981; Pang et al., 1983, 1984]; however, studies in humans have been inconsistent, some finding that age has no effect [Arendt et al., 1977, 1979, 1982; Wirz-Justice and Richter, 1979; Birau, 1981; Smith et al.,

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Address reprint requests to Dr. Robert L. Sack, Mail Code L450, Department of Psychiatry, School of Medicine, Oregon Health Sciences University, Portland, OR 97201.

1981; Wetterberg et al., 1981; Ferrier et al., 1982; Beck-Friis et al., 1984; Illnerova et al., 1985], others showing a decline with age [Touitou et al., 1981, 1984; Iguchi et al., 1982; Nair et al., 1986].

Total daily aMT production was estimated using a highly accurate, sensitive and precise assay for its major urinary metabolite, 6-hydroxymelatonin (aMT.6H). Melatonin is almost entirely metabolized in the liver to aMT.6H, which is then conjugated into the sulfate (aMT.6s) or glucuronide and excreted into the urine [Kopin et al., 1961]. Since daytime production of aMT is relatively small, an overnight urine collection provides a convenient sampling interval, and the measurement of overnight urinary aMT.6H excretion provides an integrated measure of total aMT production. We report here on aMT.6H excretion across a wide age span in a sample of healthy adults balanced for age and sex with repeated measures 6 months later.

MATERIALS AND METHODS

Subjects

Sixty subjects (30 men and 30 women) were recruited from among medical students, hospital personnel, and retirement home tenants. All subjects provided informed consent and were in good general health and drug-free for at least 1 month. Subjects were recruited in order to balance the sample for sex and age as shown in Table 1. Other demographic variables are summarized in Table 2.

Sample Collection

Each subject collected urine at home for three consecutive nights from 1700 until 1000, around the time of the summer solstice. No restrictions were imposed regarding light exposure, sleep, activity, or diet. Each subject filled out an initial health and habits questionnaire and then completed daily diaries on alcohol/caffeine consumption and sleep times for the days of sample collection. A subset of the subjects (N = 23) repeated these procedures around the time of the winter solstice. Urine samples were aliquoted and frozen for subsequent analysis.

Urinary 6-OH Melatonin Analysis

Urinary aMT.6H was measured using the gas chromatographic-negative chemical ionization mass spectrometic (GCMS) method developed by Markey and colleagues [Tetsuo et al., 1980, 1981a]. In summary, an internal standard of tetradeutero-aMT.6s is added to 3 ml of urine, the conjugates are hydro-

TABLE 1. Means and Standard Deviations of Summer 6-Hydroxymelatonin (μg/overnight 17-hr collection) by Age and Sex

	Age (years)			
Sex	20–39 (N)	40–59 (N)	60–79 (N)	80+ (N)
Male	12.1 ± 5.1 (10)	11.1 ± 7.1 (7)	6.3 ± 2.7 (8)	6.2 ± 4.4 (5)
Female	11.4 ± 5.1 (10)	6.0 ± 3.2 (8)	5.1 ± 3.5 (7)	5.8 ± 3.3 (5)
Total	11.7 ± 5.0	8.4 ± 5.8	5.7 ± 3.0	6.0 ± 3.7

TABLE 2. Demographic Features of the Study Population

	Male (N = 30)	Female (N = 30)
Smoker (%)		
Yes	13	10
No	87	90
Age (years)		
Mean ± SD	53 ± 22	53 ± 21
Range	22–94	22–87
Height (in.)		
Mean ± SD	70 ± 2	65 ± 2
Range	66–76	60–70
Diff. from ideal weight (kg)		
Mean ± SD	4.4 ± 8.0	7.7 ± 9.5
Range	−12–23	−7–28
Alcohol consumption (drinks/day)		
Mean ± SD	1.0 ± 1.0	0.3 ± 0.5
Range	0–4	0–2
Coffee (cups/day)		
Mean ± SD	2.7 ± 2.2	2.4 ± 1.8
Range	0–8	0–7
Sleep (hr/day)		
Mean ± SD	7.4 ± 0.9	7.4 ± 0.9
Range	4.5–8.5	6–9

lyzed enzymatically, and the free aMT.6H is extracted into dichloromethane. This is reacted with t-butyldimethylchlorosilane and pentafluoroproprionic anhydride, and the product is partially purified on a silica gel column. The ratio of deuterated to endogenous aMT.6H derivatives is then determined using GCMS. In our laboratory, the sensitivity is 300 pg/ml urine, and the intraassay coefficient of variation (CV) is 10.2% and the interassay CV 20.1%.

Statistical Methods

Preliminary analysis of the raw data indicated that the night-to-night variance in aMT.6H determinations was larger for the subjects with higher average aMT.6H values than for subjects with lower average values. Therefore, to improve the homogeneity of variance, we tested several variance-stabilizing transformations [$\sqrt{x}$, $\ln(x)$, and $\ln(x + 1)$] and found that, when raw aMT.6H measurements were transformed to their square roots, a satisfactory homogeneity of variance resulted. All of our subsequent analyses were therefore performed on the square root transformations.

In our analyses involving body size, we examined height, weight, and "relative obesity" (difference from ideal weight). Relative obesity was calculated by first computing an ideal weight for each subject using normative data [Bray, 1979] [for males, ideal weight (kg) = 1.88 × height (in.) −61.7; for females, ideal weight (kg) = 1.56 × height (in.) − 44.7] and then subtracting the ideal weight from the observed weight.

Winter and summer aMT.6H excretions were compared using a randomized block design; this is equivalent to a two-way analysis of variance with subjects as one factor and season as the other [Dunn and Clack, 1974]. This

enabled us to adjust for individual differences in aMT.6H excretion between subjects when testing for seasonal differences. Only summer values were used for all other analyses, because not all the subjects had both summer and winter determinations.

To assess the influence of age, sex, height, and other factors on aMT.6H, we first calculated correlation coefficients between each of these variables and average summer aMT.6H values (univariant analysis). We then employed a multiple linear regression analysis to examine the simultaneous effects of these variables on aMT.6H excretion. Finally, we compared within vs. between subject variability. To adjust for known demographic differences among the subjects, differences between observed and expected aMT.6H values were first calculated from the best-fitting model obtained from our multiple regression analysis. These differences ("residuals") were then analyzed using a one-way analysis of variance to see if the variability between subjects was significantly greater than the variability within subjects.

RESULTS

We found no evidence to suggest that total aMT production is increased in the winter. On the contrary, mean aMT levels were slightly (but statistically insignificantly) decreased in the winter (8.3 μg) relative to levels observed during the preceding summer (9.2 μg).

Next we examined the influence of demographic variables. Group means for summer overnight excretion of aMT.6H are presented in Table 1. Figure 1 shows a scattergram of the mean summer values of each subject plotted against age. These data suggest that aMT production declines with age in both men and women.

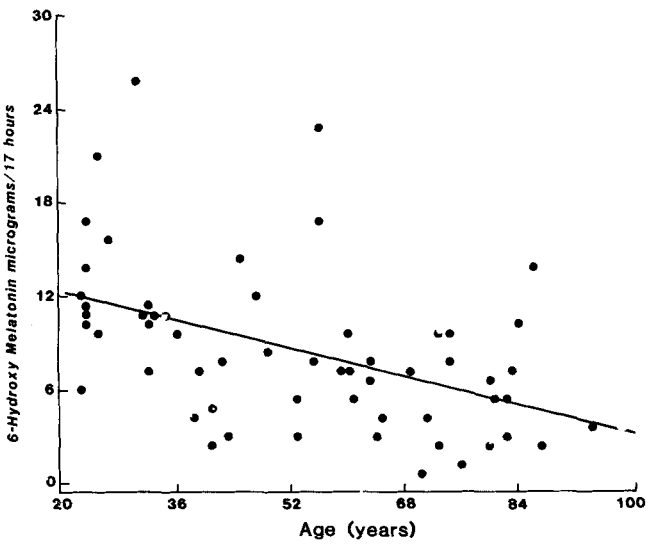

Fig. 1. Each point represents the mean of three overnight urinary 6-hydroxymelatonin determinations from a single subject. A "best fit" line is drawn through the data points showing an inverse relationship between 6-hydroxymelatonin excretion and age.

To examine the effect of other demographic variables on aMT production, we first calculated correlation coefficients between age, sex, height, weight (and other variables) and aMT.6H excretion (Table 3). On the basis of this univariate analysis, there was a strong negative correlation with age ($r = -0.47$, $P < 0.001$). There was also a positive correlation with height ($r = 0.39$, $P < 0.002$). However, when the simultaneous effects of demographic variables on aMT.6H excretion were assessed using a multiple linear regression analysis and a "best fit" model, age was still significantly associated with aMT.6H production in this model ($P < 0.0001$), but height (adjusted for age) was not (Table 4). Therefore, if the data are adjusted for age, height does not need to be taken into account. There was a negative association between aMT.6H production and relative obesity in women ($P < 0.05$) and a trend for an opposite effect in men.

Finally, using the regression model to adjust for the effects of age, sex, and relative obesity on 6-OH aMT levels, we compared within and between subject variability using a one-way analysis of variance. Even after adjustment for known demographic differences in our sample, we still found between

TABLE 3. Univariate Correlations With Square Root of aMT.6H Excretion

Variable	Corrected coefficient	Significance[4]
Age[1]	−.47	.001
Height	.39	.002
Weight	.11	.42
Relative obesity[2]	−.18	.17
Alcohol consumption (drinks/day)	.18	.16
Coffee consumption (cups/day)	−.15	.24
Sleep (hr/day)	−.01	.93
Sex[3]	−.19	.15
Smoking status	.13	.34

[1]Age = age − 51.
[2]Relative obesity = weight − ideal weight (kg).
[3]For sex and smoking status, testing for no correlation is equivalent to performing an unpaired t test.
[4]All P values are two-sided.

TABLE 4. Best Fitting Regression Model for Square Root of aMT.6H

Factor	Coefficient	SD	Significance[3]
Grand mean (square root)	2.82	.12	
Age[1]	−.020	.0047	.0001
Relative obesity[2]			
Males	.029	.016	.072
Females	−.029	.013	.026

[1]Age = age −51.
[2]Relative obesity = weight − ideal weight (kg).
[3]All P values are two-sided.

subject variability to be six times greater than within subject variability ($F = 6.3$; $df = 58$ between, 103 within; $P < 0.0001$). A test for homogeneity of variances was also significant ($P < 0.001$), however, indicating that within subject variability is not constant but varies from person to person.

DISCUSSION

Until the very recent development of an RIA [Franey et al., 1984; Griffiths et al., 1986], the only available method for measuring aMT.6H was mass spectrographic; consequently, previous studies of aMT.6H are few. Urinary aMT.6H and aMT.6s excretion parallels the circadian variation in plasma aMT concentration [Tetsuo et al., 1980a; Fellenberg et al., 1980; Markey et al., 1985]. Light exposure reduces and pinealectomy virtually abolishes plasma aMT levels and urinary excretion of aMT.6H in rats [Lewy et al., 1980; Markey and Buell, 1982] and rhesus monkeys [Tetsuo et al., 1982a]. Patients with orthostatic hypotension secondary to autonomic nervous system pathology have reduced plasma aMT levels and excrete greatly diminished amounts of aMT.6H [Tetsuo et al., 1981b], presumably reflecting a lack of sympathetic stimulation of the pineal. The phase of the menstrual cycle does not appear to affect aMT.6s excretion [Fellenberg et al., 1982].

The quantities of aMT.6H produced by our young adult subjects are similar to those previously reported. For example, Tetsuo et al. [1982b] found the average aMT.6H excretion for men and women to be 12 μg-day (range 6–19). The range of aMT.6H excretion in our total sample was quite wide. The smallest overnight aMT.6H excretion was about 2 μg and the greatest about 28 μg, a 14-fold difference. Within each age group, there were "outliers" who appeared to excrete considerably more or less aMT.6H than the average. Neither total sleep time nor caffeine/alcohol consumption appeared to affect aMT production significantly.

Surprisingly, time of the year did not appear to affect aMT production in this sample of healthy adults. Since animal studies [Illnerova and Vanecek, 1980] have shown that the winter months (short photoperiod) are associated with prolonged overnight secretion of aMT, we expected to find a larger quantity of aMT.6H produced in the winter. Several previous human studies have found evidence for seasonal variations in peak plasma aMT [Arendt et al., 1977, 1979; Wirz-Justice and Richter, 1979; Touitou et al., 1984], but the highest levels were not observed consistently during the winter months with the shortest photoperiod. Beck-Friis et al. [1984] found no seasonal differences in peak plasma aMT but found that levels remained elevated later in the morning. Recently, Illnerova et al. [1985] have found that aMT secretion begins later during the winter than during the summer (consistent with our predictions) [Lewy, 1983; Lewy et al., 1984]); however, no difference in peak levels or duration of production was found. Griffiths et al. [1986] found no change in aMT.6s in comparing the four seasons of the year in volunteers living in Antarctica but some evidence for a phase delay in the winter. Thus, in humans, it is likely that the time of year affects the timing of aMT secretion more than the amplitude.

Previously reported data on the relationship between aMT production and body size (height, weight, and obesity) are often contradictory. Beck-Friis et al. [1984] found a significant negative correlation between plasma aMT and height, whereas we found a positive correlation between aMT.6H excretion and height, which when we adjusted height for age was no longer significant. (It is possible that subjects with increased height have greater blood volume, diluting plasma aMT concentrations but not affecting urinary aMT.6H levels). We found no correlation between aMT.6H production and body weight; thus we were unable to support Arendt et al. [1982], who found a positive correlation between plasma aMT and weight for women, and Ferrier et al. [1982], who found a positive correlation for men. Beck-Friis et al. [1984] found a significant negative correlation between plasma aMT levels and obesity in a mixed sex population. Tamarkin et al. [1982] found normal plasma aMT levels in very obese children. We found a negative association between aMT.6H levels and obesity in women but an opposite association for men. Thus the relationship of aMT production to measurements of body size remains unclear. Perhaps further investigation of individuals at extremes of height and weight would be enlightening.

The demographic variable having the clearest influence on aMT production is age. Our results are in agreement with most previous reports in rodents and in man [Reiter et al., 1980a,b, 1981; Pang et al., 1983, 1984; Touitou et al., 1981, 1984; Iguchi et al., 1982; Nair et al., 1986]. Several factors could contribute to this decline. Calcification of the pineal gland is common with aging. Those rodent species that characteristically develop heavy calcifications of the pineal also have large declines in pineal aMT [Reiter et al., 1980b]. β-Adrenergic receptors on the pinealocytes decline with age in rodents [Greenberg and Weiss, 1978], suggesting another mechanism for reduced aMT production. Studies in humans, however, suggest that calcification of the pineal does not affect the histology of the pinealocyte [Tapp and Huxley, 1972] nor the activity of pineal enzyumes responsible for aMT synthesis [Wurtman et al., 1964]. In future studies, it would be of interest to correlate measures of pineal calcification with aMT production.

Metabolic alterations have to be considered in accounting for the reduction in aMT production with age. While increased clearance could account for the previously reported decline in plasma aMT with age, our finding of a reduced urinary excretion of aMT.6H makes this explanation less likely. Also, Markey et al. [1985] found a strong correlation between plasma and urinary estimates of aMT production performed simultaneously in the same subjects. Thus pineal production of aMT, not clearance, appears to be the main determinate of both plasma levels and urinary excretion.

We offer the following speculation on the significance of the decline in aMT production with age observed in this study. In general, circadian amplitude is dampened in the elderly. For example, older people have been shown to have less of the deeper stages (stages III and IV) of sleep [Feinberg, 1975] and to have less amplitude in core body temperature [Weitzman et al., 1982]. The circadian rhythm in blood testosterone is reduced in aged males [Bremner et al., 1983]. It is conceivable that the tendency for dampened circadian amplitude that accompanies aging is related to reduced levels of aMT produc-

tion; Oxenkrug et al. [1984] have shown in rats that both aging and pinealectomy result in increased morning plasma corticosterone levels with less day-night difference.

In summary, we conclude that aMT production declines with age in humans. Future studies that compare amplitudes of aMT production between groups of subjects will need to control for age. Studies correlating aMT production with measurements of body size, including height, weight, and indicators of obesity, have been contradictory, and further research is needed to clarify this relationship. Time of the year does not appear to affect amplitude of aMT production significantly, although it might have an effect on its timing.

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