Research report

Effect of photoperiod on the diurnal melatonin and 5-methoxytryptophol rhythms in the human pineal gland

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Abstract

The mammalian pineal organ is a major endocrine component of the circadian timing system, primarily concerned with the regulation of photoperiodic responses. The effect of different photoperiods on the diurnal variation in the concentration of melatonin and 5-methoxytryptophol (5-ML) was studied in the pineal glands, obtained at autopsy, of 38 human subjects, and measured by radioimmunoassay. A diurnal rhythm in pineal melatonin was evident only in the long photoperiod (April–September) with melatonin concentrations being 4.2 times higher at night (22.00–10.00 h) than during the day (10.00–22.00 h). In contrast, diurnal variations in the pineal 5-ML content were only observed in the short photoperiod (October–March) with high concentrations during day-time and low concentrations during night-time. In general, night-time concentrations of both melatonin and 5-ML were higher in summer than in winter. These data suggest that the synthesis of indoleamines in the human pineal exhibits a diurnal rhythm which is affected by seasonal changes in day length.

Keywords: Melatonin; 5-Methoxytryptophol; Pineal organ; Human; Photoperiod; Season; Circadian rhythm

1. Introduction

The pineal organ is a major endocrine component in the regulation of diurnal, menstrual and seasonal rhythmic functions [2,19,34,43,57,60]. The pineal receives environmental information through the brain and relays it to the body by means of the humoral secretion of a variety of peptides and indoleamines, such as melatonin [15,16,55,58,66]. The function of melatonin is to act as a chemical signal that conveys information about the photoperiod in the form of its circadian pattern of secretion.

In mammals the pineal production of melatonin is high during the dark phase of the 24-h light-dark cycle and low during the light phase, regardless of whether the species is active during the day or during the night, or whether it exhibits a crepuscular activity pattern [1,4,27,44,57]. This diurnal rhythm of melatonin secretion is generated in the suprachiasmatic nucleus (SCN),

at least in rats [38] and rhesus monkeys [45] and is sustained in the absence of environmental lighting information [39].

There is now considerable evidence that the diurnal melatonin signal encodes the photoperiodic message for eliciting seasonally appropriate reproductive, metabolic and behavioral responses [6,15,44,55,56]. Therefore, annual melatonin secretion has been studied under natural and artificial lighting conditions known to modulate the temporal organization of the melatonin rhythm in a variety of species, including humans.

In humans, like in other mammals, serum and urine concentrations of melatonin have been found to increase during the night and to respond to fluctuations in photoperiod and light intensity (see e.g. [3,8,14,26,29,31,59,61,62,65]). Moreover, our group recently found a diurnal rhythm in the concentration of melatonin and 5-methoxytryptophol (5-ML) in the human pineal [53]. Thus it seems that the pineal organ in humans does respond to light, although it is not well understood how its melatonin production is shaped by the photoperiod. To find out whether and how the

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pineal activity in humans is affected by day length we compared the diurnal variation in pineal melatonin and 5-ML content in two groups of subjects who had died in different periods of the year.

2. Materials and methods

2.1. Subjects

Brains of 38 human subjects (31 males and 7 females), ranging in age from 18–92 years, were obtained at autopsy. The brains were collected and neuropathologically examined either at the University of Kentucky Medical Center (UKMC), Lexington, KY (USA) or The Netherlands Institute for Brain Research (NIBR), Amsterdam during the period 1987–1988. Most of the pineal organs ($n = 32$) were obtained from people who had died in Lexington (38°N), where photoperiods range from 9 h and 30 min at the time of the winter solstice, to 14 h and 48 min at the time of the summer solstice. The rest of the pineal tissue ($n = 6$) was collected in Amsterdam at 52°N, where photoperiods vary from 7 h and 45 min to 16 h and 44 min at the corresponding times of the year. In all cases the sex and age of the patient as well as the time and cause of death were recorded. The post-mortem interval was less than 24 h (12.5 ± 1.5 h) in all but 3 subjects, whose post-mortem interval was less than 36 h. No correlation between post-mortem interval and pineal indole levels was found (linear regression analysis, $P > 0.35$ in all cases). In addition, there was no significant difference in post-mortem interval between the four groups studied (two-way ANOVA, $P > 0.4$). Thirty-one cases were accidental deaths, being caused by automotive trauma, acute cardiovascular failure, drugs and gunshot wounds. The same set of human brains has been used in a previous study in which the effects of age and Alzheimer's disease were investigated on the diurnal rhythmicity of pineal indoleamines [53].

2.2. Histology

The pineal organ was dissected from each brain and stored at -40°C (UKMC) or at -80°C (NIBR) until analysis. Pineal hormone levels were measured by radioimmunoassay (RIA) methods. For the RIA, portions of frozen pineal organs were placed in 1 ml Tricine buffer (0.1 M, pH 5, containing 0.9% NaCl and 0.1% gelatine), weighed and sonicated (10 s). The homogenates were centrifuged ($15,000 \times g$, 10 min) and the supernatant assayed for melatonin and 5-ML. The concentration of pineal melatonin was measured by RIA using melatonin antiserum 704/8483 (University of Surrey) at a final dilution of 1:16,000 [18]. Pineal 5-ML was determined by RIA using a sheep antiserum

(No. 1320) at a final dilution of 1:80,000 [52]. Both RIAs were validated for the direct measurement of melatonin and 5-ML in the human pineal by parallelism and recovery studies (for details, see [53]). Pineal melatonin and 5-ML concentrations were expressed in pg/mg wet weight of tissue.

2.3. Data analysis

In order to determine the diurnal variations in indole levels of the pineal organ subjects were grouped into two diurnal periods based on the time of death: 10.00–22.00 h and 22.00–10.00 h, since these times are known to be associated with concentration differences of melatonin and 5-ML in human plasma [1,2,36,51]. In addition, subjects were grouped into two annual periods of equal length according to a photoperiodic division of the year: 23 September–21 March ('short photoperiod') and 21 March–23 September ('long photoperiod'). At the latitude at which the study was performed day lengths vary between 9 and 12 h (weighted mean interval) for the short photoperiod and between 12 and 15 h for the long photoperiod. The distribution of the time of death of the subjects over the year and the mean vectors of the samples are given in Fig. 1. Differences in indole levels between groups were tested two-tailed using the Mann-Whitney U -test. The effect of age on melatonin and 5-ML levels in the pineal organs was evaluated statistically by two-factor analysis of variance (ANOVA). All results are expressed as means $\pm$ S.E.M. Differences were considered statistically significant at the $P < 0.05$ (two-tailed) level.

Fig. 1. Distribution of time of death of subjects over the year. Subjects were grouped into two periods of equal length according to photoperiod, indicated by the horizontal line. The mean vector length (r) and mean angle (ϕ), converted to the calendar scale, of the samples are $r_1 = 0.71$ and $\phi_1 = 15$ January for the short photoperiod and $r_2 = 0.91$ and $\phi_2 = 20$ May for the long photoperiod.

Fig. 2. The effect of photoperiod on melatonin content in human pineals during the day (10.00–22.00 h) and night (22.00–10.00 h). Values are means $\pm$ S.E.M. Only during long photoperiods (April–September) were day- and night-time melatonin levels statistically different (* $P < 0.05$). For further details see Table 1.

Fig. 3. The effect of photoperiod on 5-methoxytryptophol (5-ML) content in human pineals during the day (10.00–22.00 h) and night (22.00–10.00 h). Values are means $\pm$ S.E.M. Only during short photoperiods (October–March) were day- and night-time 5-ML levels statistically different (* $P < 0.05$). For further details see Table 2.

3. Results

3.1. Melatonin rhythm

The time of death has a major influence on the concentration of melatonin and 5-ML in the human pineal [53]. These diurnal variations in indole content, however, were found to depend on the time of year. The pineal melatonin content only showed a marked diurnal variation in subjects who had died during the long photoperiod (LP), from April to September ($P = 0.040$; Table 1 and Fig. 2). The melatonin concentration was, on average, 4.2 times higher during the night (22.00–10.00 h) than during the day (10.00–22.00 h) (360 ± 95 pg/mg and 87 ± 25 pg/mg, respectively). In contrast, there was no significant difference between the night-time melatonin concentration and the day-time values during the short photoperiod (SP), from October to March ($P = 0.35$; Table 1 and Fig. 2).

The absence of diurnal melatonin variations in the SP group was mainly due to a drop in the melatonin concentration during the night in that period of the year (Table 1). As a result, the mean melatonin levels measured over the whole 24-h period were significantly higher during the long photoperiod than during the

short photoperiod (233 ± 57 pg/mg and 103 ± 69 pg/mg, respectively; $P = 0.040$).

3.2. 5-ML rhythm

The diurnal 5-ML rhythm in the human pineal was also found to depend upon the time of year, its pattern being the reverse of the melatonin rhythm. A marked diurnal variation in the pineal 5-ML content was only observed in subjects who had died during the short photoperiod ($P = 0.028$; Table 2 and Fig. 3). The concentration of 5-ML was, on average, 7.5 times higher during the day than during the night (3.97 ± 1.53 pg/mg and 0.53 ± 0.20 pg/mg, respectively). No significant diurnal variation in 5-ML levels could be detected in the LP group ($P = 0.58$). The amount of 5-ML within each pineal was considerably less than the amount of melatonin. The median ratio of the melatonin: 5-ML concentration showed seasonal fluctuations with night-time values being 3 times higher in LP subjects than in SP subjects, whereas no difference in the day-time values between the two groups was observed.

In general, the night-time concentrations of both melatonin and 5-ML in the human pineal were lower during the short photoperiod (October–March) than during the long photoperiod (April–September). The

Table 1
Effect of photoperiod on the diurnal melatonin rhythm in the human pineal gland

Time of year	Pineal melatonin content (pg/mg) ^a		Statistics ^b
	Day (10.00–22.00 h)	Night (22.00–10.00 h)	
Short photoperiod (23 Sep–21 Mar)	50.6 $\pm$ 17.8 (5)	155.8 $\pm$ 139.8 (5)	$P = 0.347$
Long photoperiod (21 Mar–23 Sep)	86.7 $\pm$ 25.2 (13)	359.8 $\pm$ 94.7 (15)	$P = 0.040$
Statistics ^b	$P = 0.522$	$P = 0.074$	

^a Data are presented as means $\pm$ S.E.M. with the number of subjects in each group in parentheses.

^b Mann–Whitney *U*-test.

Table 2
Effect of photoperiod on the diurnal 5-methoxytryptophol (5-ML) rhythm in the human pineal gland

Time of year	Pineal 5-ML content (pg/mg) ^a		Statistics ^b
	Day (10.00–22.00 h)	Night (22.00–10.00 h)	
Short photoperiod (23 Sep–21 Mar)	3.97 ± 1.53 (5)	0.53 ± 0.20 (5)	<i>P</i> = 0.028
Long photoperiod (21 Mar–23 Sep)	3.13 ± 0.78 (13)	2.22 ± 0.41 (15)	<i>P</i> = 0.580
Statistics ^b	<i>P</i> = 0.730	<i>P</i> = 0.008	

^a Data are presented as means ± S.E.M. with the number of subjects in each group in parentheses.

^b Mann–Whitney *U*-test.

day-time values of both pineal indoles, on the other hand, did not show annual variations.

As the night-time differences in the pineal melatonin and 5-ML content between the two seasonal groups could have been due to age differences [53], the age distribution of all four groups considered in this study was investigated. No differences in age between subjects in any of these groups were detected (*P* > 0.4). Furthermore, the observed seasonal changes in the diurnal activity rhythm of the human pineal cannot be explained by differences in post-mortem delay, since no statistically significant differences were found among the groups investigated with respect to this parameter (*P* > 0.4). In addition, no correlation was found between post-mortem interval and pineal melatonin or 5-ML content (linear regression analysis, *P* > 0.35 in all cases; see [53]).

4. Discussion

Human beings are not considered to be particularly photoperiodic, although many annual rhythms have been documented in a wide variety of physiological, endocrine and behavioral processes [5,20,21,47,48,56]. Many of these annual rhythms of human biology are under environmental control and seem to be driven by an endogenous clock synchronized with the seasonal fluctuations in the 24-h light-dark cycle. Several investigations indicate that the SCN, which is considered to be the principal component of the biological clock, and the pineal are part of the neural substrate that mediates these annual rhythms [9,19,33,67].

The seasonal variation in the diurnal indole rhythms in the human pineal, as found in the present study, is in accordance with this view and with the notion that photoperiod may act as an effective zeitgeber for annual rhythms. The pineal melatonin content was found to be higher during the long photoperiod (April–September) than during the short photoperiod (October–March). In general, a clear daily rhythm in the pineal melatonin concentration was present in the summer, with high concentrations during the night-time and low concentrations during the day-time. In the winter there

was a considerable drop in the night-time values and a significant decrease in the amplitude of the diurnal rhythm. At first sight, these results seem to be counter intuitive, since a high production rate of melatonin, as the chemical signal of darkness, might be expected to occur in the winter, the time of the year with short days and low light intensities, and not in the summer. In fact that is what has been observed in nearly all mammals examined thus far, viz., that the duration of the nocturnal elevation of melatonin secretion is related proportionally to the length of the dark phase (see e.g. [2,6,10,17]).

In humans, however, there is some controversy about what aspects of the daily melatonin rhythm are used as the major seasonal zeitgeber. Existing evidence from people in their home environment indicates that extra darkness during the winter has little effect upon the total production of melatonin and the excretion of its metabolites [50], and that the timing (phase) of the melatonin rhythm, rather than its duration, is of prime importance in both temperate [11,29,32] and polar [12,54] latitudes. According to other authors [8,14,54,62,63] the duration and amplitude of the daily melatonin rhythm are also affected by seasonal changes in photoperiod. Most of these human studies, however, were performed under experimental conditions in order to simulate a natural lighting regime and did not record the circadian rhythm profiles as we experience them in our everyday artificial environment.

That the nocturnal melatonin production in the human pineal was found to be higher in the long photoperiod than in the short photoperiod adds to previous evidence for an elevated pineal activity in summer under constant environmental conditions (photoperiod, temperature and humidity), showing that the content of arginine vasotocin [41], vasopressin and oxytocin [37], number of synaptic ribbons [30] and anti-tumor activity [7] in the rat pineal are higher in summer than in winter. There also have been indications for seasonal changes in the enzymes of the pineal melatonin biosynthetic pathway under controlled conditions with peak levels in July–August [13,28]. Our findings suggest that the seasonal fluctuations in pineal activity in modern urbanized human beings, who are

living in an artificial environment, resemble that of mammals living under fixed light-dark conditions. It seems as if the extension of the natural photoperiod with artificial light to maintain long summer-type photoperiods throughout the year, which already began in prehistoric times, has significantly altered the intrinsic diurnal patterns of many physiological and behavioral functions in modern man [46,63,64].

Furthermore, it should be noted that in humans the diurnal and seasonal rhythms in pineal melatonin synthesis are inversely related to the vasopressin synthesis in the SCN [22,24,25]. The SCN contained fewer vasopressin-immunoreactive neurons during the night than during the day and showed significant fluctuations over the year with a maximum in early autumn, a lower plateau in winter, and a deep trough in late spring and early summer. In other words, the vasopressin production of the SCN is high when the pineal melatonin concentration is low and vice versa. These findings, together with evidence derived from animal studies, indicate that the synthesizing activity patterns of the SCN and pineal gland are associated and that these structures are involved in a neuroendocrine negative feedback loop (see e.g. [15,19,35,42,49,57]). The non-sinusoidal pattern of the annual cycle of the human SCN [23,24,40], however, suggests that, in addition to the environmental lighting conditions, other, non-photic signals are involved in generating seasonal cycles. Whether the seasonal melatonin rhythm in the human pineal shows a similar complex pattern remains to be determined. Finally, a word of caution is appropriate here, because it is feasible that something other than photoperiod accounts for the seasonal fluctuations in pineal indole synthesis, e.g. the well-known inter-individual variation in melatonin and 5-ML levels. Therefore more human pineals need to be examined to confirm these interesting findings.

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