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Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease

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Melatonin and 5-methoxytryptophol (ML) were measured in human pineals (38 controls, 16 subjects with Alzheimer's disease). Time of death had a major influence on the indole concentrations with significantly higher melatonin levels occurring at night (22.00–10.00 h) and significantly higher ML levels occurring during the day (10.00–22.00 h). This daily variation disappeared in both the older subjects (55–92 years) and in the Alzheimer patients (55–89 years).

The 5-methoxyindoles, melatonin and 5-methoxytryptophol (ML) are primarily synthesized in the mammalian pineal gland. Melatonin has been detected in the human pineal by gas chromatography (GC)⁶, GC-mass spectrometry (GC/MS)⁴, high pressure liquid chromatography (HPLC)¹ and radioimmunoassay (RIA)¹⁶. These studies have reported a wide variation in the concentration of melatonin (<0.5–400 ng/pineal), but the relationship between melatonin content and sample (sex, age, disease state) or post-mortem (time of death, post-mortem interval) variables has not been well clarified.

The rhythm of melatonin is generated in the suprachiasmatic nucleus (SCN), at least in rats⁹ and rhesus monkeys¹⁰. Changes in the SCN (SCN cell number, arginine-vasopressin cells) have been shown in old age and Alzheimer's disease^{18,19}. Whether these SCN alterations affect the content/rhythm of melatonin in the human pineal gland is not yet known.

A RIA which was developed to measure pineal ML in several species¹⁴ has recently been validated to measure ML in human plasma¹². A low-amplitude daily variation in ML was found in plasma taken from healthy volunteers. Whether a similar pattern of ML occurs in the human pineal has not yet been determined.

The aim of the present study was to assess which factors, i.e. time and cause of death, sex and age of the subject, post-mortem interval, affect the concentration of

melatonin and ML in the human pineal gland. In addition, a group of Alzheimer patients were studied to investigate the effect of this disease on pineal melatonin and ML levels.

Human pineal glands were obtained at autopsy from two collection centres, the University of Kentucky Medical Center, Lexington, Kentucky, U.S.A. (Centre A) and The Netherlands Institute for Brain Research, Amsterdam (Centre B) during 1986–1988. In all cases the sex and age of the patient as well as the time and cause of death were recorded.

At Centre A, pineal glands were collected from 32 control individuals (27 males, 5 females) ranging in age from 18 to 76 years (45 ± 3 years). The post-mortem interval was less than 24 h (14.2 ± 1.1 h) in all but 3 subjects, whose post-mortem interval was less than 36 h. Thirty-one cases were accidental deaths. The causes being automotive trauma ($n = 6$), sudden cardiovascular causes ($n = 8$), accidental gunshot wounds ($n = 5$), drugs ($n = 3$) and ethanol ($n = 1$) overdoses, asphyxia ($n = 2$), peritonitis ($n = 1$), transected brainstem ($n = 1$) and liver disease ($n = 1$). The cause of death of 4 suicide victims was gunshot wounds.

At Centre B, pineal glands were obtained from 6 controls (4 males, 2 females; age range 60–92 years) and 16 Alzheimers patients (6 males, 10 females; age range 55–89 years). In all cases ($n = 22$), the post-mortem interval was less than 6.5 h (4.3 ± 0.2 h).

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Pineal tissue was stored between -40 and -80 °C until analysis.

For the RIA, portions of frozen pineal glands 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 g, 10 min) and the supernatant assayed for ML and melatonin.

Pineal ML was measured by RIA¹⁴ using a sheep antiserum (batch no. 1320) at a final dilution of 1:80,000. Previous studies show low cross-reactivity between the antiserum and a range of indoles; the greatest cross-reactivity (0.73%) occurring with 5-methoxyindole¹⁴. The minimum detection level of the assay, defined as the amount of ML producing a displacement of 2 S.D. from maximum binding, was 1 pg/tube.

The concentration of pineal melatonin was determined by RIA⁵ using melatonin antiserum batch no. 704/8483 (University of Surrey) at a final dilution of 1:16,000. The primary cross-reactants were N-acetyltryptamine (0.97%), 6-hydroxymelatonin (0.38%) and N-acetyltryptophan (0.26%). The limit of sensitivity of the assay, defined as 2 S.D. of maximum binding, was 2.5 pg/tube.

Both RIAs were validated for the direct measurement of ML and melatonin in the human pineal by parallelism studies. Pooled human pineal homogenates serially diluted with assay buffer gave displacements parallel to that of the ML and melatonin standard, respectively. Recovery of ML was also assessed by adding standard ML (8 and 20 pg) to the samples.

All results were expressed as means $\pm$ S.E.M. Pineal melatonin and ML concentrations were expressed in pg/mg wet weight of tissue. Pineal melatonin values were divided into two time periods: 10.00–22.00 h and 22.00–10.00 h, since these times are known to be associated with basal (10.00–22.00 h) or elevated (22.00–10.00 h) levels of melatonin in human plasma². Pineal ML concentrations were similarly subdivided as increased levels of ML have been found between 10.00–22.00 h in human plasma¹².

The control pineal group consisted of the non-Alzheimer pineals obtained from both collection centres (Centres A and B). The effect of age, sex and post-mortem interval on day- and night-time melatonin and ML levels in the control pineals was assessed using the unpaired Student's *t*-test and linear regression analysis.

In our study the content of melatonin in the control pineals ($n = 38$) was significantly higher between 22.00 and 10.00 h ($n = 20$) than between 10.00 and 22.00 h ($n = 18$) ($P < 0.05$) (Fig. 1A). The mean % increase was 530%. ML was detected in all but 6 control pineal glands (5 of which were collected during the night). The concentration of ML in the pineals was significantly

higher during the day (10.00–22.00 h) than during the night (22.00–10.00 h) ($P < 0.05$) (Fig. 1B). The mean % increase was 190%.

The amount of ML within each pineal was less than the amount of melatonin. The mean ratio ($\pm$ S.E.M.) of melatonin: ML was 58 ± 24 during the day and 172 ± 52 during the night. In order to verify that the low levels of ML measured in the pineal were not a result of procedural losses, a recovery study was performed. Recovery of ML (8 and 20 pg) from pineal homogenates was 83.4 ± 4.4 and $83.3 \pm 3.9\%$ (mean $\pm$ S.E.M., $n = 7$ each), respectively.

The effect of age on melatonin and ML content in the control pineals was evaluated. Linear regression analysis failed to show any correlation between melatonin or ML levels and the age of the subject at death ($P > 0.2$ in all cases). The day- and night-time melatonin and ML values were also divided into two age groups (18–54 and 55–92 years, $n = 21$ and 17 respectively) and compared. The mean melatonin and ML content in each age group is shown in Fig. 2A and 2B, respectively. The daily variation in melatonin and ML was maintained in the younger age group (18–54 years) with significantly higher melatonin concentrations at night ($P < 0.05$) and higher, although not significant ($P < 0.09$), ML levels during the day. In contrast there was no significant difference between the day-time melatonin and ML concentrations

Fig. 1. (a) Melatonin and (b) 5-methoxytryptophol levels in 38 control human pineal glands during the day (10.00–22.00 h, $n = 18$) and night (22.00–10.00 h, $n = 20$). Values are means $\pm$ S.E.M. ** $P < 0.05$ compared with 22.00–10.00 h group.

and the night-time values in the 55–92 years age group ($P > 0.25$ in both cases).

The effect of sex of the subject on day- and night-time melatonin and ML was also assessed. Day- and night-time melatonin levels were not significantly different between males (day-time 60 ± 14 pg/mg, $n = 14$; night-time 425 ± 172 pg/mg, $n = 17$) and females (day-time 136 ± 67 pg/mg, $n = 4$; night-time 317 ± 208 pg/mg, $n = 3$). Likewise males and females had similar day-time (males 2.8 ± 0.7 pg/mg, $n = 14$; females 5.4 ± 1.9 pg/mg, $n = 4$) and night-time (males 1.9 ± 0.4 pg/mg, $n = 17$; females 1.5 ± 1.2 pg/mg, $n = 3$) ML concentrations.

No correlation between post-mortem interval and pineal melatonin or ML content was found (linear regression analysis, $P > 0.2$ in all cases). Although day- and night-time melatonin and ML levels were reduced in pineals having a post-mortem interval greater than 8 h, the difference was not significant ($P > 0.3$ in all cases).

It was not possible to determine whether the cause of death affected the melatonin and ML content in the control pineals since, once time of death and age were controlled for, the number of subjects/cause of death was too small. However, the pineal melatonin and ML content of the 4 suicide victims (39 ± 11 years) who had died of gunshot wounds was compared directly with 3 subjects (25 ± 9 years) who had died of accidental

Fig. 2. The effect of age on (a) melatonin and (b) 5-methoxytryptophol (ML) content in control human pineals during the day (□) and night (▨). Values are means $\pm$ S.E.M. The number of subjects in each group is shown in the bars. * $P < 0.1$, ** $P < 0.05$ compared with the age-matched night-time group.

Fig. 3. Day-time (□) and night-time (▨) pattern of pineal (a) melatonin and (b) 5-methoxytryptophol in controls and Alzheimer's patients. The number of subjects in each group is shown in the bars. Values are means $\pm$ S.E.M. * $P < 0.1$, ** $P < 0.05$ compared with the night-time control group.

gunshot wounds at the same time of day (11.00–20.00 h). Although the suicide group had lower melatonin (27 ± 12 pg/mg) and higher ML (4.6 ± 1.9 pg/mg) concentrations than the non-suicide controls (158 ± 91 pg/mg melatonin; 3.9 ± 2.4 pg/mg ML), the differences were not statistically significant.

Of the Alzheimer patients studied, 8 died during the day and 8 died during the night. The concentrations of pineal melatonin and ML in the Alzheimer group were compared with those of the non-Alzheimer control group, (Fig. 3A and 3B, respectively). The marked daily variation in melatonin and ML disappeared in the Alzheimer group, the Alzheimer sufferers having significantly reduced melatonin levels ($P < 0.05$) and higher, although not significant, ML levels during the night compared with the controls. The night-time concentrations of melatonin and ML in the Alzheimer patients were similar to their day-time levels and the day-time levels observed in the control group. As the night-time changes in melatonin and ML observed in the Alzheimer group could have been due to the large age difference between the 2 groups (Alzheimer group 79 ± 3 years, $n = 8$; control group 47 ± 4 years, $n = 20$), the night-time melatonin and ML levels of the Alzheimer subjects were compared with those of control subjects in the same range (65 ± 1 years, $n = 8$). Once age-matched, no significant difference in the night-time pineal melatonin

and ML content between the 2 groups was observed.

This study shows a daily variation in the concentration of melatonin and ML in the human pineal gland. Significantly higher melatonin levels were measured in subjects who had died during the night and significantly higher ML levels were measured in subjects who had died during the day. This pattern is consistent with the reported rhythm of melatonin² and ML¹² in human plasma. Moreover, elevated night-time melatonin levels have previously been observed in the human pineal^{6,16}.

For the first time a day/night pattern in human pineal ML content has been observed, its daily variation being the inverse of the melatonin pattern. A similar rhythm in ML has previously been found in the pineal glands of Syrian hamsters^{3,13} and box turtles¹⁵.

Within a single pineal, the concentration of ML was 50- to 200-fold less than the concentration of melatonin. These low amounts of ML, however, do not seem to be the result of breakdown after death or during analysis. In addition, the amount of pineal ML measured in our study (0.1–13.8 pg/mg) agrees closely with the levels reported by Beck et al.⁴ (0.8–17.8 pg/mg).

Age of the subject had a significant effect on the day/night variation of melatonin and ML, the rhythmicity being lost in the older age group. This decline in the night-time production of melatonin with age agrees with previous reports^{7,11,20,21}. Although the physiological significance of this age-dependent reduction is not yet known, the disappearance of the melatonin and ML rhythm coincides with the general loss of circadian rhythms in senescence and the degeneration of the SCN^{18,19}.

A recent publication by Stanley and Brown¹⁶ reported that suicide victims had reduced pineal melatonin con-

centrations at night (22.00–06.00 h) compared with controls. The suicide group in our study was closely matched with a control group for age, cause of death and time of death. Consistent with the findings of Stanley and Brown¹⁶, although not significant, melatonin was reduced. Investigation of a larger number of subjects at different times of the day, however, may reveal significant differences.

Patients who had Alzheimer's disease had significantly reduced melatonin levels and significantly increased ML levels during the night compared with the non-Alzheimer group. This loss of rhythmicity in the Alzheimer group agrees with previous reports showing a strong loss of circadian rhythms and cells in the SCN of Alzheimer patients^{18,19}. When more accurately age-matched controls were compared with the Alzheimer subjects, no differences between the two groups were observed. This finding strengthens, in addition, the argument for accurate age-matching when investigating abnormalities in the human brain¹⁷.

Jengeleski et al.⁸ have found abnormal noradrenergic axons in the pineals taken from Alzheimer sufferers and old controls compared with young controls. Like in the present study, no distinction between the effect of age and Alzheimer's disease could be made. In order to see whether Alzheimer changes in the pineal are different, or more pronounced than those observed in normal aging, future investigations require more young Alzheimer patients and old controls. Only then may the relative contribution of age and disease be discerned.

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