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5-Methoxytryptophol and melatonin in children: Differences due to age and sex

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Abstract. It seems clear that the pineal hormone, melatonin (N-acetyl-5-methoxytryptamine), is involved in the reproductive behavior of several animal species including humans. Moreover, several data also support a role for 5-methoxytryptophol (ML), another pineal hormone, in the control of sexual processes. To test the role of ML in human reproductive axis, 128 healthy children, 68 boys and 60 girls, were studied. Each of these groups was divided in three age subgroups of 6, 11, and 14 years. A single blood sample (0900 hours) was obtained from each subject to determine melatonin, ML, FSH, LH, estradiol (girls), and testoterone (boys) by RIA. Statistical analysis of the data included ANOVA-II (factor I: age, factor II: sex) and an analysis of covariance with age as covariate. A similar plasma melatonin concentration, with a significant decrease between 6 and 11 years, was found in boys and girls. Melatonin concentrations correlate well with initiation of the pubertal development in these children, although no sex differences were found. Concentrations of ML are approximately 50% of those of melatonin. In contrast to melatonin, ML levels show significant age and sex differences. Plasma ML concentration significantly increased in boys ($P < 0.001$) and decreased in girls ($P < 0.001$) after 8 years of age. These results support the hypothesis that, besides melatonin, other pineal compounds such as ML may be involved in the maturation process in humans. The pineal indole ML may also be used as a marker of the different chronobiology in the pubertal development in boys and girls.

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Introduction

Puberty is a complex process involving pulsatile secretion of gonadotropin-releasing hormone (GnRH) [Sizonenko and Auber, 1986; Grumbach and Kaplan, 1989]. The hypothalamic GnRH pulse generator receives neural and humoral signals including sexual steroid, metabolic, and environmental inputs. It is not known if the GnRH pulse generator neurons have intrinsic pulsatile properties or if the GnRH pulsatility depends on a primary impulse (by neurotransmitters and/or neuromodulators) to these neurons [Grumbach and Kaplan, 1989; Kelch et al., 1990].

One of these neuromodulators involved in pubertal development may be the pineal hormone melatonin (N-acetyl-5-methoxytryptamine, melatonin). The pineal gland is a part of the photoneuroendocrine pathway that, beginning in the retina, transduces the environmental light in an endocrine secretion. Pinealocytes, similar to other cells, transform tryptophan to 5-hydroxytryptamine. However, pinealocytes specifically produce melatonin from 5-hydroxytryptamine. Although the best known pineal hormone is melatonin, this gland may secrete several other hormones such as indoles (derived from the 5-hydroxytryptamine metabolism) and peptides [Cardinali, 1981]. In the human pinealocyte at least five different active methoxyindoles exist [Beck et al., 1982].

Three of the 5-methoxyindoles synthesized by the pineal gland, i.e., melatonin, 5-methoxytryptophol (ML), and 5-methoxytryptamine, show a photoperiod-dependent production. In some animal species it was demonstrated that the amplitude of the daily rhythms of melatonin and ML is significantly modi-

fied throughout the year. Besides, melatonin and ML are very responsive to the experimental manipulation of the photoperiod [Skene el al., 1986; Vivien-Roels et al., 1993]. Both compounds show a circadian rhythm, melatonin peaking at 0200 hours and ML peaking at 1100 hours [Pévet et al., 1981; Skene et al., 1990; Vivien-Roels et al., 1993].

In seasonal breeding animals the pineal acts by synchronizing the reproductive functions to the best environmental conditions. Thus, ovulation takes place in some of these animal species during spring [Tamarkin et al., 1979; Goldman, 1983]. About 30 years ago it was unsuccessfully attempted to prove a similar regulatory gonadal function by the pineal gland in humans. However, other melatonin functions, such as its role in regulating the sleep/awake cycle [Dollins et al., 1994; Dawson and Encel, 1993], in synchronizing biological rhythms [Deacon et al., 1994], and in modulating brain excitability [Champney and Peterson, 1993; Molina-Carballo et al., 1994a,b; Acuña-Castroviejo et al., 1995], are present both in animals and in humans. The recent reported role of melatonin as a free radical scavenger [Reiter et al., 1995] and its ability to inhibit brain nitric oxide synthase [Pozo et al., 1994] open a new perspective in the study of the role of melatonin in gonadotropin regulation.

The endocrine effects of ML are very similar to those of melatonin [Reiter, 1989; Pévet, 1995]. ML has been implicated more closely than melatonin in the reproductive control of some mammals [Pévet et al., 1981]. Therefore, ML also might play a role in human reproduction. The availability of a specific RIA for ML [Pévet, 1983; Skene et al., 1989] has permitted the study of this hormone during changes in the human's reproductive axis, mainly during puberty. We considered it worthwhile to study the changes in melatonin and ML in humans with the follow objectives: a) to determine the levels of melatonin and ML in healthy children from 6 to 14 years of age (prepubertal and pubertal children), and b) to analyse the relationships between these hormones and their potential roles in sexual maturation.

Materials and methods

A total of 128 children was included in a routine program of infantile health carried out in a rural village of Jaén (Spain). The study was done in the Spring (March), and the blood samples were taken at 0900 hours while the subjects were fasting. Samples were obtained for a routine analysis (hemogram, cholesterol, and triglycerides). The remaining plasma was used to measure the hormonal parameters of this study. The sommatometric characteristics of each child (weight, size, and skin folds) and the blood pressure were recorded.

The children selected for this study satisfied the following criteria: a) lack of familiar antecedents of congenital illness, b) absence of known familiar and/or personal illness antecedents of interest, c) normal psychomotor and somatometric development, and d) normal clinical and routine biochemical findings and absence of medication during the study. Informed consent was obtained from all parents and from the hospital's Ethical Committee, in accordance with the declaration of Helsinki of 1975, as revised in 1983.

Blood samples were centrifuged at 3,000g for 10 minutes, and plasma was separated and frozen at -20° C until assay. Hormonal analysis included determinations of FSH, LH, estradiol, and testosterone using commercial RIA kits (Sorin). FSH and LH were determined in unextracted serum. FSH MRC 78/549 and LH MRC 68/40 were used as the standard for the FSH and LH RIAs, respectively. Estradiol determinations were carried out following serum extraction with ether [Fernández et al., 1990]. Testosterone was determined directly by RIA without extraction. Quality controls of the RIAs were done. Sensitivity, i.e., the minimum amount of hormone different from zero, was FSH: 1 mUI/ml; LH: 1 mUI/ml; testosterone: 0.04 ng/ml; estradiol: 4.5 pg/ml. Intra- and inter-assay coefficients of variation for the hormone RIAs were as follows: FSH: 1.9, 1.9%; LH: 2.4, 3.8%; testosterone: 7.2, 10.2%; and estradiol: 8.0, 7.9%.

Plasma melatonin levels were measured in duplicate using a commercial RIA kit (WHB, Bromma, Sweden), as described previously [Fernández et al., 1990]. In control samples, the intra- and inter-assay coefficients of variation for melatonin RIA were 11.3 and 16.3%, respectively. The recovery of added melatonin was 84%, and the sensitivity of the assay was 5 pg/ml. Plasma ML levels were measured in duplicate using a commercial antibody (Stockgrand, Ltd., Surrey, Guildford) and 125I-ML as tracer (DuPont NEN). The protocol used in ML RIA was the provided by the antibody manufacturer. The intra- and inter-assay coefficients of variation for ML RIA were 9.6 and 13.2%, respectively. The recovery of added ML was 87% and the sensitivity of the assay was 2.5 pg/ml.

Statistics

Because the site at which the study took place (a rural school center), the degree of pubertal development (Tanner stages) could not be tested except for those individuals who were 6 years of age. The children were initially divided in three groups ac-

cording to their age as follows (Table 1): a) 6-year-old group (6 year group), including children aged between 6 and 8 year old (n = 34, 19 boys and 15 girls, mean of age 6.4 ± 0.3 years, mean ± SEM); b) 11-year-old group (11 years group), including children aged between 10 and 13 year old (n= 62, 31 boys and 31 girls, mean of age 11.2 ± 0.2 years), and c) 14-year-old group (14 year group), grouping children aged between 13 and 15 years old (n = 32, 18 boys and 14 girls, mean of age 14.1 ± 0.3 years).

Table 1 shows the hormonal profiles of FSH, LH, estradiol (girls), and testosterone (boys) of the studied children. Surprisingly, all girls in the 6-year-old group had estradiol values above 15 pg/ml, although they had no pubertal signs. One possible explanation for this high estradiol concentration could be the seasonal variation in the normal concentration of sexual steroids, implying an increase in sexual steroids in girls during spring. We cannot classify the stage of pubertal development in these children according their hormonal profiles because only one blood sample was taken.

Any children in the 6-year-old group displayed clinical signs of precocious puberty, suggesting that at this age all of them were under prepubertal stage. Moreover, all children of 7 years (girls and boys) included in the study were without the initial pubertal development. To further study any change in melatonin and ML in pre and pubertal stages, we did a second classification of the children in two age groups (Table 2): a) <8-year-old group including children aged 6–7 year old (n = 33, 18 boys, and 15 girls; 22.87 ± 0.87 and 23.08 ± 3.31 kg of body weight, boys and girls, respectively), and b) >8-year-old group, including children aged between 10 and 15 year old (n= 95, 50 boys and 45 girls; 45.42 + 12.08 and 45.68 + 10.78 kg of body weight, boys and girls, respectively). This classification is supported by the following: a) the normal pubertal development is initiated in girls 2–3 years before that in boys, and never before 8 years of age, and b) the significant decrease in melatonin levels that was reported to be related with pubertal development takes place 2–3 years before physical signs of puberty appear.

Data are expressed as mean ± SEM. Statistical analysis (BMDP) includes a two-way ANOVA (factor I: age; factor II: sex), and analysis of covariance, with age as covariate.

Results

Age-dependent changes in plasma levels of melatonin and ML in boys and girls are shown in Figure 1. ANOVA analysis showed significant changes for these indoles with age ($F = 16.03$, $P < 0.001$ for melatonin; $F = 22.00$, $P < 0.001$ for ML). Plasma melatonin levels significantly decreased from 6 to 11 years in boys ($P < 0.001$). In girls the decrease of melatonin no reached statistical significance ($P < 0.1$). Interestingly, melatonin levels increased from 11 to 14 years, reaching similar values to those found in the 6 year group. However, no significant melatonin differences between sex were found in the age groups.

Absolute ML levels are the 50% of those of the melatonin. Moreover, whereas the melatonin concentrations are similar in girls and boys for each age group, the levels of ML show significant differences between sexes. In this respect, the ML levels in boys are approximately one half of the concentration of the indole in girls ($P < 0.001$) in the 6 year group (Fig. 1). This relation is reversed in the 11 year group, with ML levels being significantly higher in boys compared to girls ($P < 0.001$). Age-dependent changes of ML are also different in girls and in boys. This indole significantly increased from 6 to 11 years in boys ($P < 0.001$), showing no significant changes from 11 to 14 years. However, in girls ML significantly decreased from 6 to 11 years (21.18 ± 3.42 vs. 11.97 ± 2.18, $P < 0.001$). Moreover, this indole significantly increased from 11 to 14 years in girls ($P < 0.001$), reaching in this group a values similar to that in boys.

Figure 2 shows melatonin and ML values comparing the pre- and pubertal stages in the studied children. No changes in melatonin concentrations were found in relation to sex, but significant differences were noted between the two age groups ($P < 0.01$). In both sexes, melatonin decreased at puberty onset.

The behavior of ML is different from melatonin.

Table 1. Plasma levels of FSH (mUI/ml), LH (mUI/ml), estradiol (pg/ml), and testosterone (pg/ml) in boys (M) and girls (F) grouped by age

Hormone	6 years	11 years	14 years	F	P
FSH (M)	0.55 ± 0.10	1.40 ± 0.13	3.50 ± 0.66	15.03	< 0.001
FSH (F)	1.18 ± 0.14	4.29 ± 0.35	5.15 ± 1.41	7.51	< 0.01
LH (M)	0.24 ± 0.03	1.14 ± 0.20	3.06 ± 0.44	22.31	< 0.001
LH (F)	0.20 ± 0.04	2.48 ± 0.47	8.31 ± 3.31	5.91	< 0.01
Estradiol (F)	22.34 ± 2.03	34.72 ± 3.08	87.92 ± 14.41	22.02	< 0.001
Testosterone (M)	0.25 ± 0.14	0.51 ± 0.23	5.74 ± 0.55	81.08	< 0.001

M, Male; F, female.

Table 2. Plasma levels of FSH (mUI/ml), LH (mUI/ml), estradiol (pg/ml), and testosterone (pg/ml) in boys and girls by in only two age groups: <8 years old and >8 years old.

	<8 year old		>8 year old	
Hormone	Boys (n = 18)	Girls (n = 15)	Boys (n = 50)	Girls (n= 45)
FSH	0.55 ± 0.44	2.3 ± 2.17	1 28 ± 0.56	4 59 ± 3.33
LH	0.24 ± 0.14	1.96 ± 1.78	0.21 ± 0.18	4 52 ± 7.97
Estradiol	—	—	22.34 ± 7.85	51.28 ± 41 0
Testosterone	0.25 ± 0.64	2.43 ± 3.08	—	—

Between the groups with less o greater eight years, the differences obtained for ML concentrations are sex-dependent. In boys, ML increased in the > 8 year group compared to < 8 year group ($P < 0.001$). However, in girls ML significantly decreased > 8 year group compared to < 8 year ($P < 0.01$) (Fig. 2). Whereas ML levels are higher in girls in the younger children ($P < 0.01$), in older children the levels of the indole are higher in boys ($P < 0.001$).

To further study the variations of melatonin and ML according to age, a covariance analysis with data grouped by sex, and age as covariate was performed. The results of this analysis show that the concentrations of melatonin, but not of ML, significantly covary with age ($F = 22.16$, $P < 0.001$)

Discussion

The participation of melatonin and ML in reproduction in non-human animals is clear [Pévet, 1983], although differences between relative importance of melatonin and ML have been reported [Tamarkin et al., 1979; Reiter, 1980; Goldman, 1983; Pévet, 1983]. In humans, modifications of pineal function are associated with pubertal alterations (either premature or delayed puberty), and abnormally high melatonin levels are associated with hypothalamic hypogonadism [Berga et al., 1988]. However, there are no studies involving ML and the hypothalamus-pituitary-gonadal axis in humans. Only one study in growth delayed children reported changes in ML in prepubertal and pubertal patients has been reported [Hooper et al., 1979]. Thus, to our knowledge, this is the first study showing the evolution of plasma ML concentration in normal boys and girls before and after their pubertal development, and its comparison with the changes in melatonin during the same period. It was not possible to determine the Tanner stage of pubertal development in our study, but the simultaneous measurement of testosterone, estradiol, FSH, and LH allowed us to classify the children according to the known distribution of the degree of pubertal development in each age group.

The presence of GnRH pulses is responsible for pubertal onset [Sizonenko and Auber, 1986; Grumbach and Klapan, 1989]. After birth, the GnRH pulse generator is active until 6 and 12 months of life in boys and girls, respectively [Kelch et al., 1990; Forest, 1989]. From this age to 6–9 years, the intrinsic inhibitor of GnRH pulse genera-

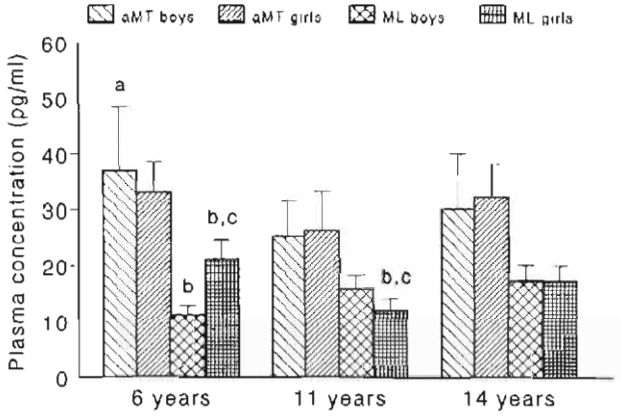

Fig. 1. Changes in melatonin and ML concentrations in boys and girls grouped by age. **a**: Significant differences in melatonin levels in boys between 6 and 11 year of age ($P < 0.001$). **b**: Significant differences in ML levels between 6 and 11 year groups in boys and girls, and between 11 and 14 year groups in girls ($P < 0.001$). **c**: significant differences in ML levels between boys and girls in each group ($P < 0.001$).

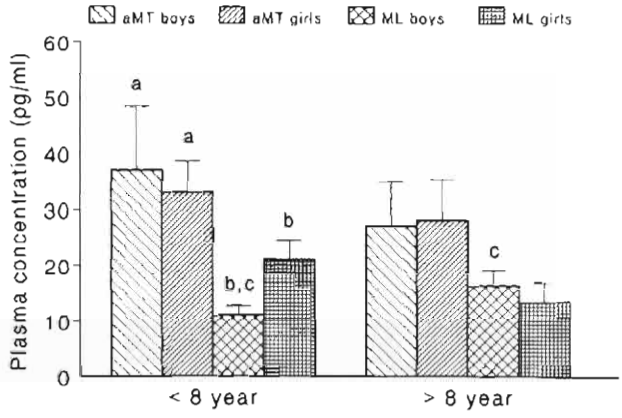

Fig. 2. Changes in melatonin and ML concentrations in boys and girls grouped in only two age groups (<8 year old and >8 years old). **a**: $P < 0.05$ vs. >8 year group. **b**: $P < 0.001$ vs. >8 year group. **c**: $P < 0.001$ between boys and girls in < 8 year group.

tor is operative. Between 6 and 9 years of age, the efficiency of the intrinsic inhibition of GnRH pulsatility decreases, resulting in an increase in gonadotropins and in gonadal steroid secretion [Sizonenko and Aubert, 1990]. The mechanisms responsible for these hypothalamic responses, although a multifactorial origin, are not totally known.

In the human, it was proposed that melatonin might be an important factor responsible for prepubertal inhibition of the hypothalamic GnRH pulse generator [Silman, 1991; Waldhauser et al., 1984, 1991]. High doses of melatonin (75 mg), sufficient to maintain high nocturnal melatonin levels, are effective in inhibiting the GnRH pulse generator [Silman, 1993]. Recently, hypogonadism with high levels of melatonin in a patient with a hyperplasic pineal was reported. Pubertal development in this patient only began when the abnormally high levels of circulating melatonin decreased [Puig-Domingo et al., 1992]. Thus, the rapid decrease of melatonin during the prepubertal period, and the lower levels of this hormone thereafter, may facilitate pubertal onset [Silman et al., 1979; Gupta et al., 1983].

The theory of the pineal-related pubertal onset [Silman et al., 1979] is based on the fact that 1) pineal melatonin secretion remains constant during childhood but 2) body weight increases, producing a dilution of melatonin levels such that plasma concentration of melatonin decreases. When melatonin levels during its acrophase (0200 hours) decrease below a critical value the GnRH hypothalamic pulse generator is released from its inhibition. A lack of pineal growth during childhood was recently reported [Schmidt et al., 1995], further supporting the "dilution theory" of melatonin action. Also, the existence of a time-coincidence between the maximum of melatonin secretion (nocturnal melatonin peak) and its efficacy in inhibiting the GnRH pulse generator was reported [Silman et al., 1979]. Consequently, this mechanism may come into play when the infant displays his own circadian rhythm of melatonin [Attanasio et al., 1986]. In the newborn, the pineal gland is active but not rhythmic [Jaldo et al., 1993; Muñoz et al., 1992, 1993], with the endogenous melatonin rhythm beginning between 3 and 6 months of life [Attanasio et al., 1986]. Thus, the lack of GnRH inhibition during the first months of life in infants could depend, at least partially, on the lack of a melatonin circadian rhythm at this age. Thereafter the melatonin appears, and its nocturnal peak may inhibit GnRH pulsatility until puberty, when melatonin levels decrease.

The relationship between melatonin and pubertal development in children at different ages and Tanner stages have been studied [Cavallo, 1991, 1992; Cavallo et al., 1992]. The authors reported that the nocturnal melatonin peak decreases with age and pubertal development. However, the authors did not find a relation between melatonin and puberty when the data were analysed with age as a covariate. Our results show that when melatonin data are grouped by sex, melatonin levels covary with age.

Gupta et al. [1983] did not find changes in daytime melatonin levels in children during pubertal development. However, when the net increments (night vs. day values) in melatonin were examined, a significant decline was observed from pubertal stage I to II [Gupta et al., 1983]. Moreover, Attanasio et al. [1983] showed that the day-night increment in melatonin levels was related to maturation stage, subjects with precocious puberty having increments lower than the aged-matched controls, and subjects with delayed puberty showing increments comparable to those of preschool children. The blood samples in our study were obtained at 0900 hours, a day time at which pineal melatonin production is low. Nevertheless, a significant decrease in plasma melatonin levels from 6 to 11 years in both sexes was noted, consistent with reports [Waldhauser et al., 1984; Attanasio et al., 1985]. From 11 to 14 years, plasma levels of melatonin increased in both sexes. The origin of this increase in melatonin after puberty was not clear, although a delay in the melatonin rhythm acrophase might increase the melatonin levels measured at morning. Moreover, when melatonin levels were studied in terms of pubertal development, significant differences between the indole concentrations before and after puberty were found. These data suggest a role for melatonin in pubertal changes.

Our results show lower ML concentrations than melatonin reported previously [Skene et al., 1989; Carter et al., 1979]. One interesting finding in ML levels is the different behavior of this indole with sex and age, in comparison with the changes shown for melatonin. In boys, the ML levels increased from 6 to 14 years. In girls the ML levels showed a biphasic behavior, decreasing from 6 to 11 years and then increasing to 14 years, reaching similar values at this age as in boys. In the < 8 year group, the ML concentration is higher in girls compared to boys. From < 8 year group to > 8 year group, ML levels increased in boys and decreased in girls, reaching similar values in both sexes.

The ML data suggest a role for this indole in human reproduction. However, if most of the girls included in the 14 year group were in an advanced stage of puberty, why where ML concentrations increased in this group from the minimum reached in the 11 year group? It is possible that the decrease in ML levels between 6 and 11 years has a permissive effect on the pubertal onset in girls. Once ini-

tiated, pubertal processes cannot regress, and the subsequent rises in ML levels between 11 and 14 years could have some other significance, such as an increase in the production of the indole or a delay of its acrophase. Nevertheless, we give importance to the fact that (in girls) when the data are grouped in only two groups, the ML concentrations are significantly lower in the older group.

The increase in ML in boys from 6 to 11 years suggests a sex dependent behavior of this indole. Rather than being an antigonadotropic hormone, perhaps ML has a progonadotropic activity. The balance between melatonin and ML might be a factor involved in normal pubertal development. Nevertheless, the results suggest that ML might be a useful marker for the different chronology of pubertal development in each sex. Owing to ethical considerations we only took one blood sample (0900 hours) from each child, making it impossible to study the circadian rhythm of ML. However, in the few animal species including humans [Beck et al., 1982; Skene et al., 1986] in which ML was measured, it seems to display a circadian rhythm, similar to melatonin [Vivien-Roels et al., 1993]. However, the circadian rhythm of ML differs from the melatonin rhythm in that the acrophase of the ML rhythm occurs at 1100 hours [Carter et al., 1979; Skene et al., 1986; Hofman et al., 1995]. This hour coincides with the time of the day at which the samples were taken in our study. Although the human being is not a seasonal reproductive mammal, it is important to remember the data regarding the geographic location and the season of the year at which the study was done. Our study was performed in the spring in the south of Spain, with a photoperiod of 13 hours light and 11 hours darkness. In any season of the year plasma concentrations of ML are higher during the day, when the NAT activity is decreased. Changes in NAT activity are responsible for changes in the pineal serotonin metabolism, with acetylation predominating during the night (producing melatonin), and oxidation during the day (producing ML) [Mefford et al., 1983].

In summary, the data present here reinforce the theory that pineal hormonal secretions in addition to melatonin are involved in the maturation. The data must be confirmed in other studies; one such study should be the determination of the circadian rhythm of ML and its changes with age and pubertal development classified according Tanner stages and sexual hormonal profiles. Such a study (in advanced elaboration by our workgroup) may explain the differences found in the concentration and evolution of ML levels between girls and boys, and would allow a more scientifically-based hypothesis regarding the possible role of ML in human puberty.

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