J Neural Transm (1996) 103: 833–842

Journal of Neural Transmission

Pineal response after pyridoxine test in children

A. Muñoz-Hoyos[1], **I. Amorós-Rodríguez**[1], **A. Molina-Carballo**[1], **J. Uberos-Fernández**[1], and **D. Acuña-Castroviejo**[2]

[1] Departamento de Pediatría, Hospital Universitario, Facultad de Medicina, and [2] Instituto de Biotecnología, Universidad de Granada, Spain

Accepted February 6, 1996

Summary. To characterize the pineal response to pyridoxine, plasma melatonin was measured in one hundred and twenty children 3 hours after vitamin B_6 administration. The children, aged between 1.5 and 8 years, were divided in four groups as follows: a) control day group, grouping 27 children sampled at 9:00 and at 12:00; b) control night group, grouping 29 children sampled at 21:00 and at 24:00; c) pyridoxine day group, grouping 30 children sampled at 9:00, then intravenously (i.v.) injected with 3 mg/kg of pyridoxine, and sampled at 12:00; and d) pyridoxine night group, grouping 34 children sampled at 21:00, i.v. injected with 3 mg/kg of pyridoxine, and sampled at 24:00. Melatonin concentration was measured by radioimmuno assay. The data obtained showed a significant increase in melatonin levels after pyridoxine administration in the pyridoxine night group (39.87 ± 8.02 pg/ml basal vs 88.45 ± 9.21 pg/ml after pyridoxine, $p < 0.001$). The other groups did not showed significant differences in melatonin concentrations. Statistical analysis shows that the administration of pyridoxine during the nocturnal hours represents a stimulating factor to increase the pineal production of melatonin in children.

Keywords: Melatonin, pyridoxine, children, pineal function test, circadian rhythms

Introduction

Melatonin production by the pineal gland displays a circadian rhythm peaking at night in all animal species studied including man (Cardinali, 1981; Reiter, 1986; Vivien-Roels and Pevet, 1993). This rhythm is under photoperiodic regulation (Humlová and Illnerová, 1992), with light decreasing and dark increasing N-acetyltransferase activity, the limiting enzyme of melatonin synthesis in the pineal gland (Klein, 1978). Pinealocyte uses tryptophan as substrate for melatonin synthesis, and melatonin levels change as a function of tryptophan availability. In sheep, Namboodiri (1983) reported an increase in pineal melatonin production after hydroxytryptophan administration. More recently, Huether et al. (1992) and Yaga et al. (1993) showed that tryptophan

loading increases daytime serum melatonin levels in rats, either from pineal and extrapineal origin. Huether et al. (1993) also showed an elevation in serum melatonin levels after serotonin-releasing drugs administration in rats.

The relationships between vitamin B_6 and endocrine system have been previously showed (Rose, 1978). It is well known the activity of pyridoxine as a coenzyme in the tryptophan metabolism, both in the kynurenine and in the methoxyindole pathways (Klein et al., 1980; Quay, 1980). In the methoxyindole pathway, pyridoxine acts as coenzyme of the 5-hydroxytryptophan decarboxylase. The enzyme decarboxylates 5-hydroxytryptophan, yielding 5-hydroxytryptamine (serotonin), the immediate precursor of melatonin (Klein et al., 1980; Quay, 1980). Thus, the vitamin B_6 deficience may induce important metabolic alterations that involve pyridoxine-dependent enzymes. Coenzymatic activity of pyridoxine exerts important roles not only in tryptophan metabolism but also in neurotransmitter biosynthesis (Dolina et al., 1993). In this respect the convulsive manifestations that appear during pyridoxine deficiency are well known (Acuña et al., 1994; Bessey et al., 1957; Hellström and Vasella, 1962; Sherman, 1954). Moreover, the pineal hormone melatonin increases brain pyridoxal phosphokinase activity (Anton-Tay and Sepúlveda, 1970) and exerts important inhibitory effects on the CNS activity. These inhibitory effects of melatonin involve an increase GABA-benzodiazepine receptor complex activity and an inhibition of glutamatergic neurotransmission (Acuña-Castroviejo et al., 1986a,b, 1990, 1993, 1994, 1995). The effects of melatonin may explain the relation between melatonin and convulsions supported by the literature (Anton-Tay, 1974; Champney and Petterson, 1993; Molina et al., 1994a,b). The existence of the relationships between tryptophan metabolism, melatonin synthesis and rhythmicity, and brain activity, prompt us to evaluate the participation of pyridoxine in the dirunal and nocturnal melatonin production.

Subjects and methods

Subjects

The study was carried out in 120 children at the University of Granada Hospital (Granada, Spain). 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. The study was done in pediatric patients aged between 1.5 and 8 years. All selected children satisfied the following criteria: a) lack of familiar antecedents of congenital illness; b) absence of known personal organic illness antecedents, excepting the typical infantile diseases with favourable evolution; c) the children were in the hospital because of non endocrine and non psychological or neurological diseases. This banal pathology included acute gastroenteritis, upper airway respiratory afections with bad oral tolerance and minor surgery, all of them with favourable progression; d) short hospital stay, between 2 and 3 days; e) normal psychomotor and somatometric development; f) normal clinical and routine biochemical findings and absence of medication during the study, which was done just one day before the patient leaves the hospital.

Study protocol

The children were divided in two groups, namely control and pyridoxine-treated ones. Furthermore, each of these groups was divided in two subgroups, diurnal and nocturnal

groups, to study the daily melatonin changes in these periods. Diurnal groups consisted of patients sampled at 9:00 and 12:00, and nocturnal groups consisted of patients sampled at 21:00 and 24:00. The inclusion of a patient in the respective subgroup only depended on the time of sample.

The control group (C) contained 56 children (47% of the total). This group was divided into two subgroups: a) control day group, comprising 27 children sampled at 9:00 (C9) and at 12:00 (C12), and b) control night group, comprising 29 children sampled at 21:00 (C21) and at 24:00 (C24).

The pyridoxine-treated group (P) contained 64 children (53% of the total). This group was divided into two subgroups: a) pyridoxine-treated day group, comprising 30 children sampled at 9:00 (P9). In this group a 15 ml isotonic saline solution containing 3 mg/kg body weight of pyridoxine was slowly (20 min) intravenously injected. The injection started immediately after 9:00 sampling. This group was newly sampled at 12:00 (P12); b) pyridoxine-treated night group, comprising 34 children, sampled at 21:00 (P21) and, after the same pyridoxine protocol as the P9 group, the children were sampled at 24:00 (P24).

The antecubital vein was cannulated in all children, and blood samples were taken to do routine biochemical analysis. The antecubital intravenous cannula was also used in our study to inject the pyridoxine dose. Two blood samples were obtained from each child, i.e. basal and three hours after. After blood centrifugation at 3,000 <g for 10 min, plasma was separated and frozen at −20°C until assay. The study was carried out along the year, and a similar number of samples were obtained for each season. Due to the geographic area in which the study was done (Granada, in the south of Spain), high intensity of daylight (when the children were sampled) is common along the year (>2,500–3,000 lux), whereas during the night light intensity was less than 200 lux always. Environmental stress was minimal due to both the banal pathology of the studied children and the time in which the samples were taken, i.e., before to withdraw the intravenous cannula.

The concentration of plasma melatonin was determined by radioimmuno assay (WHB, Bromma, Sweden). The method was validated for the direct measurement of melatonin in the human plasma by quality control studies (Fernández et al., 1990). Pooled human plasma serially diluted with assay buffer gave displacements parallel to those of melatonin standards. The intra- and interassay coefficients of variation were 11.3% and 16.3%, respectively. Recovery of melatonin, as assessed by the standard addition method, gave a value of 84.4% and sensitivity was 5 pg/ml.

Statistical analysis

All results are expressed as mean ± SEM. Plasma melatonin is expressed in pg/ml. The statistical analysis of the results included mean comparisons, Pearson's regression and correlation analysis, and Fisher's transformation.

Results

Figure 1 shows the mean ± SEM of the melatonin values in the different studied groups. In the control day group, melatonin levels oscillate from 40.39 ± 11.26 pg/ml at 9:00 (C9 group) to 36.82 ± 9.01 pg/ml at 12:00 (C12 group) (n.s.). These results are similar to those found in the pyridoxine-treated day group, which show melatonin values of 42.29 ± 9.94 pg/ml at 9:00 (P9 group) and 36.70 ± 9.23 pg/ml at 12:00 (P12 group) (n.s.).

During the nocturnal period, there are not significant changes in melatonin levels in the control night group, changing from 51.25 ± 10.54 pg/ml at 21:00 (C21 group) to 54.37 ± 10.17 pg/ml at 24:00 (C24 group) (n.s.). However, in the pyridoxine-treated night group, there is a significant increase in melato-

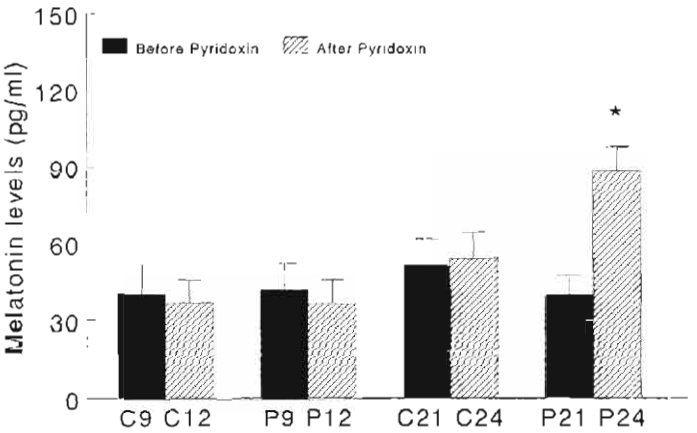

Fig. 1. Melatonin levels before and after pyridoxine administration. See Materials and methods for the legends. *P < 0.001

nin concentrations, raising from 39.87 ± 8.02 pg/ml at 21:00 (P21 group) to 88.45 ± 9.21 pg/ml at 24:00 (P24 group) ($p < 0.001$).

An interesting analysis in this experimental design was to compare the melatonin values obtained in the basal sample with those obtained 3 hours after for each group (Table 1). The results of this statistical analysis suggest the following statements: a) in the control day group there is a statistically significant correlation between melatonin levels found at 9:00 and at 12:00, with both significant correlation ($r = 0.66$, $p < 0.001$) and determination ($R = 0.44$, $p < 0.05$) coefficients. This significant correlation disappeared in the pyridoxine-treated day group ($r = 0.15$, n.s.). Moreover, during the nocturnal period there are statistically significant relationships both in the control night group ($r = 0.82$, $p < 0.001$) and in the pyridoxine-treated night group ($r = 0.41$, $p < 0.05$). The Fisher's z transformation of the correlation coefficients demonstrates different population of data, both in the day groups ($z = 7.2$, $p < 0.001$) and in the night groups ($z = 9.12$, $p < 0.001$) (Table 2).

Table 1. Correlation and regression studies between melatonin levels before and after pyridoxine administration. **R** determination coefficient

Group	r/p	R/p	Regression equation
Control day group	0.66**	0.44*	y = x/(0.66 + 0.17x)
Control night group	0.82**	0.67**	y = 12.3 + 0.99x
Pyridoxin-treated day group	0.15	0.02	—
Pyridoxin-treated night group	0.41*	0.17	y = 6.42 + 0.38x

* $p < 0.05$; ** $p < 0.001$

Table 2. Correlation coefficient analysis by Fisher's z transformation of the control group and pyridoxin-treated group

Group	z_1	z_2	SDz	z	p
Day	0.80	0.15	0.09	7.2	**
Night	1.16	0.43	0.08	9.12	**

** $p < 0.001$

Discussion

Although the pyridoxine requirements are not exactly known, it is accepted that doses between 0.5 and 1.5 mg/day are enough to basal necessities (Horwitt, 1986; Otto et al., 1957). These amounts of vitamin B_6 are found in normal diet. However, during the treatment of vitamin B_6 deficiency or in some status such as neonatal seizures, sideroacresic anemias, homocystinuria or familiar xanturenic aciduria, the doses of pyridoxine required to counteract these processes are between 10 and 100 mg/day (Cruz and Rodriguez, 1973; Escriver, 1960; Snyderman et al., 1961; Yess et al., 1964).

The absence of clinical manifestations of vitamin B_6 deficiency and the normality of the biochemical analysis support normal pyridoxine levels in the children included in our study. To perform the pyridoxine test to evaluate the pineal function, we chose a therapeutic vitamin B_6 dose. However, due to the large difference between lower and higher doses of pyridoxine used in clinic, we used a calculated dose related to body weight (3 mg/kg body weight). The dose of pyridoxine was intravenously administered in a continuous infusion during 20 min. This way of administration avoids the retard of intestinal absorption and hepatic metabolisation of pyridoxine typical of oral administration. Moreover, because the children had an antecubital intravenous cannula routinely putted in place at their admission in the hospital, we used this venous way to inject the pyridoxine dose and to take the blood samples elsewhere. Consequently, we avoid any extra stressful situation to children. Although the span of ages of patients included in the study is rather wide, we feel that this factor did not affected to the results here described. In fact, all children had finished their breast milk feeding period and thus they had the typical melatonin rhythmic secretion. Moreover, all the children were in prepubertal status, when the melatonin secretion is maximal and the regulatory mechanisms were not affected by hormones of the hypothalamus-hypophyseal-gonadal axis (Attanasio et al., 1985). Thus, the circadian rhythm of melatonin in the studied children was similar in all of them.

There are many data regarding relationships between vitamin B_6 and central nervous system, and between vitamin B_6 and several physiological processes such as nutrition, development and endocrine system (Coburn, 1994; Otto et al., 1957; Rose, 1978). However, few studies involving vitamin B_6 and melatonin exist to date (Amorós, 1994). Vitamin B_6-dependent enzymatic systems, including those involved in neurotransmitter biosynthesis (Dolina et al., 1993) and tryptophan metabolism (Klein et al., 1980; Quay, 1980), use pyridoxal-5-phosphate (the biologically active form of vitamin B_6) from the

general organic pool. In a situation of pyridoxal deficience, each enzyme will consume this cofactor according to its dissociation constant, thus allowing a variable use of the vitamin.

Koskiniemi et al. (1985) have studied the effect of an oral dose of 2g of tryptophan on patients with different neurologic pathology, including 7 cases of progressive myoclonic epilepsy. The authors measured the cephalospinal ventricular and lumbar fluid content in tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Koskiniemi et al. (1985) reported an increased availability of brain 5-hydroxytryptamine, and an increased synthesis of several kynurenines including quinolinic acid. This kynurenine is a neurotoxin involved in several neurologic alterations such as temporal lobe epilepsy, and it might exert its toxic effects acting as a potent exitatory amino acid agonist. In sheep, intraperitoneal administration of 5-hydroxytryptophan is followed by an important rise in melatonin (Namboodiri et al., 1983). The increase in melatonin levels was significantly lower after administration of tryptophan than 5-hydroxytryptophan (Namboodiri et al., 1983).

These data suggest the posible use of 5-hydroxytryoptophan as a test for pineal function in humans. However, Cavallo et al. (1987) did not found significant changes in melatonin levels 3 hours after a morning (8:00) or afternoon (14:00) oral administration of a low dose of 5-hydroxytryptophan to health children (5mg/kg body weight) and adults (10mg/kg body weight). In a previous study, we found a significant increase in melatonin levels 3 hours after a night (21:00) but not after a morning (9:00) oral administration of 20mg/kg body weight of L-5-hydroxytryptophan to normal children (Moreno, 1994). The above results suggest that both the dose and time of day of tryptophan administration are critical to induce significant changes in melatonin production. Moreover, a significant increase in urinary kynurenine metabolites was found after tryptophan administration to healthy newborns at 9:00 but not at 21:00 (Narbona et al., 1994). Our results show an increased melatonin secretion when vitamin B_6 was administered during the night hours, but it was without effect when this compound was given during the day. These results must be analysed in the light of the circadian variation in the factors involved in the production of melatonin N-acetyltransferase, hydroxy-indol-O-methyltransferase, pineal β-adrenoceptors) and in the melatonin rhythm itself (Cardinali, 1981). Nevertheless, the differences between nocturnal and diurnal pineal responses to pyridoxine suggest the existence of intracellular mechanisms not well known, but clearly related to the induction of pyridoxine-dependent enzymes acting on tryptophan metabolism. Administration of tryptophan (Narbona et al., 1994), 5-hydroxytryptophan (Moreno, 1994) or pyridoxine (Amorós, 1994) increase kynurenine metabolites when they are given at morning, and increased melatonin production after their administration at night. These effects may be explained by the increase of substrate availability and/or the activation of pyridoxine-dependent enzymes involved on tryptophan metabolism. The results also suggest that the diurnal low activity of pineal N-acetyltransferase does the pineal gland unable to synthesize melatonin during the morning hours. In this situation, tryptophan

or pyridoxine load deviate tryptophan metabolism to kynurenine pathway. However, during the night, when the pineal N-acetyltransferase activity is increasing, the pineal gland is able to synthesize melatonin, thus potentiating the tryptophan metabolism towards melatonin production.

It is important to consider that whereas tryptophan administration increases the substrate availability to synthesize serotonin and melatonin, also might induce a status of pyridoxine deficience due to pyridoxine use by the enzymes. Some pyridoxine-dependent enzymes, mainly the enzymes acting on the last steps of these metabolic pathways, may then be inactive due to the excessive consumption of pyridoxine in the first steps of the metabolism. Regarding with this possibility, it would be very interesting to do a stimulating test with pyridoxine plus tryptophan to study their effect on pineal metabolism during a more long time.

Our data further support the earlier suggestion of Bessey et al. (1957) regarding the useful of the daily administration of 5–10 mg of pyridoxin in children with idiophatic convulsive pathology. Moreover, data from our laboratory have demonstrated an increase in melatonin secretion during a seizure episode (Molina et al., 1994a,b), and repetitive seizures may lead to a pyridoxin deficiency. The increased melatonin secretion in epileptic patients treated with therapeutical doses of pyridoxin may improve the convulsive pathology. This possibility is supported by a recent report demonstrating the useful of melatonin administration in two cases of untreatable epilepsy (Champney et al., 1995). The anticonvulsant properties of melatonin may involve its recently demonstrated antioxidant effect (Reiter et al., 1995), and effect that might be potentiated with the pyridoxin administration. Thus, melatonin may delays the clinical appearence of the oxidative stress pathologies (Reiter et al., 1994).

Our results also may explain the effectiveness of several pyridoxin-based compounds used in the treatment of behavior alterations such as physical and/or psychological fatigue, memory deficits and school delay. All of these alterations may be due to a bad sleepness quality, and at least in one case, the school delay was counteracted by melatonin circadian rhythm normalisation (Tomoda et al., 1994). Recently it was showed that the sleep/wake cycle is mainly regulated by melatonin, being this indole more important than serotonin to this function (Dollins et al., 1994). Consequently, due to the sleep-induced effects of melatonin, and the melatonin-induced role of pyridoxin, the pyridoxin-based medication must be administered only before to sleep.

Lee et al. (1988) found an increased brain serotonin after tryptophan load in animals; this effect was potentiated by pyridoxin coadministration. However, pyridoxin was without effect when it was coadministered with a diet with basal tryptophan requirements. Since high tryptophan doses are used in some phsychiatric diseases, the effectiveness of these treatments may improve with the coadministration of pyridoxin. The coadministration of pyridoxin plus tryptophan will increased the levels of serotonin and then the levels of melatonin, that may be the responsibles for the therapeutical benefits of these treatment.

Acknowledgements

This study was partially supported by the Junta de Andalucía and the Hospital Universitario of Granada (Spain).

References

Acuña-Castroviejo A, Lowenstein P, Rosenstein R, Cardinali DP (1986a) Diurnal variations of benzodiazepine binding in rat cerebral cortex: disruption by pinealectomy. J Pineal Res 3: 101–109

Acuña-Castroviejo D, Rosenstein R, Romeo H, Cardinali DP (1986b) Changes in gamma-aminobutyric acid high affinity binding to cerebral cortex membranes after pinealectomy or melatonin administration to rats. Neuroendocrinology 43: 24–31

Acuña-Castroviejo D, Fernández B, Del Aguila CM, Gomar MD, Castillo JL (1994) La glándula pineal y los mecanismos de inhibición en el sistema nervioso central. In: Muñoz-Hoyos A, Fernández García JM, Acuña-Castroviejo D (eds) Aspectos morfofuncionales y fisiopatológicos de la glándula pineal. Consideraciones de interés pediátrico. Nuevas Creaciones Médicas, Madrid, pp 165–216

Acuña-Castroviejo D, Escames Rosa G, Macías González M, Muñoz Hoyos A, Molina Carballo A, Montes Ramírez R, Vives Montero F (1995) Neuroprotective role of melatonin and its therapeutical applications. J Pineal Res 19: 57–63

Anton-Tay F (1974) Melatonin: effects on brain function. Adv Biochem Psychopharmacol 11: 315–324

Anton-Tay F, Sepúlveda J, González S (1970) Increase on brain pyridoxal phosphokinase activity following melatonin administration. I. Life Sci 9: 1283–1288

Amorós I (1994) Valoración de la función pineal (doble vertiente: metoxi-indoles y kynurenina) tras una sobrecarga con piridoxina. Thesis, Universidad de Granada

Attanasio A, Borrelli P, Gupta D (1985) Circadian rhythms in serum melatonin from infancy to adolescence. Endocrinology 61(2): 388–390

Bessey OA, Adam DJD, Hansen AE (1957) Intake of vitamin B_6 and infantile convulsions: a first approximation of requirements of pyridoxine in infants. Pediatrics 20: 33–44

Cardinali DP (1981) Melatonin: a mammalian pineal hormone. Endocr Rev 2: 327–346

Cavallo A, Richards GE, Meyer WJ, Waldrop RD (1987) Evaluation of 5-hydroxytryptophan administration as a test of pineal function in humans. Horm Res 27: 69–73

Champney TH, Peterson SL (1993) Circadian, seasonal, pineal, and melatonin influences on epilepsy. In: Yu HS, Reiter RJ (eds) Melatonin: biosynthesis, physiological effects and clinical applications. CRC Press, BocaRaton, pp 477–494

Champney TH, Sánchez Forte A, Muñoz Hoyos A, Molina Carballo A, Moreno Madrid F, Acuña Castroviejo D (1995) Anticonvulsant effects of melatonin in humans: two case studies. Soc Neurosci (Abstr): 1964

Coburn SP (1994) A critical review of minimal vitamin B_6 requirements for growth in various species with a proposed method of calculation. In: Litwack G (ed) Vitamins and hormones, vol 48. Academic Press, San Diego, pp 259–300

Cruz M, Rodriguez F (1973) Metabolismo del triptófano. Arch Pediatr 141: 505–516

Dolina S, Peeling J, Sutherland G, Pillay N, Greenberg A (1993) Effect of sustained pyridoxine treatment on seizure susceptibility and regional brain amino acid levels in genetically epilepsy-prone BALB/c mice. Epilepsia 34: 33–42

Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH (1994) Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. Proc Natl Acad Sci 91: 1824–1828

Escriver CR (1960) Vitamin B_6 dependency and infantile convulsions. Pediatrics 25: 62–74

Fernández B, Malde JL, Montero D, Acuña D (1990) Relationship between adenohypo-

physeal and steroid hormones and variations in serum and urinary melatonin levels during the ovarian cycle, perimenopause and menopause in healthy women. J Steroid Biochem 35: 257–262

Gomar MD, Castillo JL, Del Aguila CM, Fernández B, Acuña-Castroviejo D (1993) Intracerebroventricular injection of naloxone blocks melatonin-dependent brain [^{3}H]flunitrazepam binding. NeuroReport 4: 987–990

Gomar MD, Fernández B, Del Aguila CM, Castillo JL, Luna JD, Acuña-Castroviejo D (1994) Influence of the behaviorally active peptides $ACTH_{1-10}$ and $ACTH_{4-10}$ on the melatonin modulation of ^{3}H-flunitrazepam receptor binding in the rat cerebral cortex. Neuroendocrinology 60: 252–260

Hellstrom B, Vasella F (1962) Tryptophan metabolism in infantile spasm. Acta Pediatr 51: 665–673

Horwitt MK (1986) Interpretations of requirements for thiamin, riboflavin, niacin-tryptophan and vitamin E plus comments on balance studies and vitamin B_6. Am J Clin Nutr 44: 973–85

Huether G, Poeggeler B, Reimer A, George A (1992) Effect of tryptophan administration on circulating melatonin levels in chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Sci 51: 945–953

Huether G, Poeggeler B, Adler L, Rüther E (1993) Effects of indirectly acting 5-HT receptor agonists on circulating melatonin levels in rats. Eur J Pharmacol 238: 249–254

Humlová M, Illnerová H (1992) Entrainment of the rat circadian clock controlling the pineal N-acetyltransferase rhythm depends on photoperiod. Brain Res 584: 226–236

Klein CD (1978) The pineal gland. A model of neuroendocrine regulation. In: Reichlin S, Baldessarini RJ, Martin JB (eds) The hypothalamus. Raven Press, New York, pp 303–327

Klein CD, Auerbach DA, Namboodiri MAA, Wheler GHT (1980) Indole metabolism in the mammalian pineal gland. In: Reiter RJ (ed) The pineal gland, vol I. Anatomy and biochemistry, CRC Press, Boca Raton, pp 200–226

Koskiniemi M, Laakso J, Kuurne T, Laipio M, Harhonen M (1985) Indole levels in human lumbar and ventricular cerebrospinal fluid and the effect of L-tryptpphan administration. Acta Neurol Scand 71: 127–132

Lee NS (1988). Dietary pyridoxine interaction with tryptophan or histidine on brain serotonin and histamine metabolism. Chem Behav 29: 559–564

Molina Carballo A, Acuña Castroviejo D, Rodriguez Cabezas T, Muñoz Hoyos A (1994a) Effects of febrile and epileptic convulsions on daily variations in plasma melatonin concentration in children. J Pineal Res 16: 1–9

Molina Carballo A, Muñoz Hoyos A, Rodriguez Cabezas T, Acuña Castroviejo D (1994b) Day-night variations in melatonin secretion by the pineal gland during febrile and epileptic convulsions in children. Psychiat Res 52: 273–283

Moreno Madrid F (1994) Valoración de un test de función pineal en niños con l-triptófano por vía oral. Thesis, Universidad de Granada

Narbona López E, Rodriguez Cabezas T, Moreno Madrid F, Uberos Fernández J (1994) Metabolismo del triptófano. Su importancia en patología infantil. In: Muñoz Hoyos A, Fernández García JM, Acuña Castroviejo D (eds) Aspectos morfofuncionales y fisiopatológicos de la glándula pineal. Consideraciones de interés pediátrico. Nuevas Creaciones Médicas, Madrid, pp 115–140

Narbona E, Maldonado J, Del Castillo ML, Bayés R, Robles C, Nuñez J, Molina JA (1984) Estudio del metabolismo del triptófano en niños afectos de epilepsia. An Esp Ped 20: 361–367

Namboodiri MAA, Sugden D, Klein DC, Mefford IN (1983) 5-Hydroxy-tryptophan elevates serum melatonin. Science 221: 569–661

Otto, Bessey OA, Adam DJD, Hansen AE (1957) Intake of vitamin B_6 and infantile

convulsions: a first approximation of requirements of pyridoxine in infants. Pediatrics 20: 33–44

Quay WB (1980) General biochemistry of the pineal gland in mammals. In: Reiter RJ (ed) The pineal gland, vol 1. Anatomy and biochemistry. CRC Press, Boca Raton, pp 173–198

Reiter RJ (1986) Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals. J Neural Transm 21: 35–54

Reiter RJ, Menéndez-Peláez A, Poeggeler B, Tan DX, Pabos MI, Acuña-Castroviejo D (1994) The role of melatonin in the pathophysiology of oxygen radical damage. In: Moller M, Pevet P (eds) Advances in pineal research, vol 8. John Libbey, London, pp 403–412

Reiter RJ, Melchiorri D, Sewerynek E, Barlow-Walden L, Chuang JI, Ortiz GG, Acuña-Castroviejo D (1995) A review of the evidence supporting melatonin's role as antioxidant. J Pineal Res 18: 1–11

Rose DP (1978) The interactions between vitamin B_6 and hormones. Vitamins and Hormones 36: 53–99

Sherman H (1954) Pyridoxine and related compounds. Effects of deficiency in animals. In: Sebrel WH, Harris RS (eds) The vitamins, vol 3. Academic Press, New York, pp 265–276

Snyderman SE, Boyer A, Phansalkar SV, Holt LE (1961) Essential aminoacids requirements of infants. Am J Dis Child 102: 163–167

Tomoda A, Miike T, Uezono K, Kawasaki T (1994) A school refusal case with biological rhythm disturbance and melatonin therapy. Brain Dev 16: 71–76

Vivien-Roels B, Pévet P (1993) Melatonin: presence and formation in invertebrates. Experientia 49: 642–647

Yaga K, Reiter RJ, Richardson BA (1993) Tryptophan loading increases daytime serum melatonin levels in intact and pinealectomized rats. Life Sci 52: 1231–1238

Yess N, Price JM, Brown RR, Swan PB, Linkswiller H (1964) Vitamin depletion in man: urinary excretion of tryptophan metabolites. J Nutr 84: 229–236

Authors' address: A. Muñoz-Hoyos, Departamento de Pediatría, Facultad de Medicina, Avda. de Madrid, 11, E-18071 Granada, Spain.

Received July 28, 1995