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Neuroendocrine and circadian aspects (melatonin and β-endorphin) of atopic dermatitis in the child

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Atopic dermatitis (AD) is a disease of increasing incidence among paediatric patients. Among the factors involved in its pathogenesis is the alteration of the immune response, and so the objective of this study was to evaluate the involvement of certain neuroendocrine factors with immune properties in the development of the disease. Fifty-five subjects were selected and divided into the following three groups: healthy subjects, those diagnosed with symptomatic AD and those with asymptomatic AD. Plasma levels of melatonin and β-endorphins were measured by radioimmunoassay, in serum samples obtained at 9 AM and 9 PM, with two samples being obtained from each of the patients and controls. In the phases of AD outbreaks, there is a reduction in the serum levels of both melatonin and β-endorphin. In the case of melatonin, the difference is statistically significant only during the day, although nocturnal levels are greater for both hormones. In AD, a central neuroendocrine dysfunction may be a primary pathogenic event. Our hypothesis is that the physiological nocturnal peak of melatonin due to pineal gland production may mask the decline of melatonin of possibly extrapineal (immunological) origin during episodes of dermatitis outbreaks. Further studies are required, particularly of neurovegetative and hormonal aspects, to better define this process. Such a definition would also be of therapeutic interest.

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Atopic dermatitis (AD) is the cutaneous expression of a disorder known as atopia, a term that was introduced in 1923 to designate a group of complaints, such as asthma and hay fever, that arise spontaneously in individuals with a family history of predisposition to the disease. These problems mainly start during the first years of life, with up to 90% of cases first appearing before the age of 5 yr (1, 2).

Although the causes of AD are as yet unexplained, various factors are known to influence the appearance of the disease and its symptoms; these include constitutional factors (genetic, immunological, etc.) that are affected by other causative elements (climatic, environmental, psychological, etc.; 2, 3).

Among the factors involved in the development of the disease is the malfunctioning of the immune response among the subjects affected. AD is an inflammatory, immunologically mediated skin disease of biphasic evolution, provoked by the dysregulation of Th1/Th2 immune responses, which results in increased immunoglobulin (Ig) E production. The acute phase of AD is characterized by a T-helper type 2 cell-predominant phenotype (partly because of the enhanced apoptosis of Th1 cells), reflected in the increased frequency of allergen-specific T cells

producing interleukin (IL)-4, -5 and -13, and by a decrease in interferon (IFN)-c-producing T cells. IL-4 is involved in IgE isotype switching, while IL-5 attracts eosinophils and prolongs their survival, which may result in the peripheral blood eosinophilia and increased IgE serum levels observable in many AD patients (4). The T-helper type 1 cell phenotype predominates during the chronic eczematous phase (5, 6). Circulating IL-16 (natural ligand levels of CD4; 7) and the concentration of soluble CD30 (8) are significantly higher in patients with AD, and IL-16 decreases significantly after topical treatment with corticosteroids or tacrolimus.

The goal of the present study was to contribute to our knowledge of the factors involved in the pathogenesis of the disease, in the area termed by Ader as Psycho-neuro-immunology (9, 10), a new discipline that studies the close links between the immune and the nervous systems. Melatonin provides a homeostatic link between the brain and the immune system. Both membrane and nuclear melatonin receptors are present in lymphocytes, and it has recently been shown that exogenous melatonin can counteract the inhibitory effect of prostaglandin E_2 (PGE_2) on IL-2 production via its aMT1 membrane receptors (11). Furthermore, there is evidence that melatonin, via nuclear receptors, is mainly responsible for most of its immune effects (12). Melatonin has specific high affinity-binding sites on both Th1 and Th2 helper cells. The present study examines the role played by two neuroendocrine mediators (melatonin and β-endorphin), which share immunological properties, in the development of the disease.

Materials and methods

Fifty-five children were selected and studied. The subjects were aged between 4 months and 5 yr, with an average age of 2 yr and 7 months. Of these children, 31 (56.36%) were girls and 24 (43.64%) were boys (Table 1). Prior informed consent was obtained from the parents for this study, carried out at the Paediatrics Service of the Hospital Ciudad de Jaén on subjects referred to the Emergency Department at this hospital from Health Centres in the region and from other hospitals in the same province.

The patients were divided into the following three groups: group A, the control group, made up of 15 healthy children; group B, comprising 20 children with AD, who at the time of the study were suffering a severe attack and group C, with another 20 children, who at the time of the study were in an asymptomatic, stable phase of the disease. For inclusion in groups B or C, we used the earlier defined criteria for atopy (13), plus the previous appearance of two severe outbreaks.

Table 1. Descriptive statistic of age and somatometric characteristics of the subjects included in the study, classified by group and gender

Group	Sex		Mean	s.d.	Min.	Max.
A	Male (n = 5)	Age	40.4	18.2	11	58
		Weight	14.6	2.4	10.5	17.0
		Height	95.2	12.1	75	105
	Female (n = 10)	Age	36.1	17.3	7	60
		Weight	14.9	3.7	8.0	21.0
		Height	94.6	14.3	70	110
B	Male (n = 7)	Age	22.0	10.8	13	44
		Weight	11.7	2.5	9.9	17.0
		Height	82.4	8.6	74	100
	Female (n = 13)	Age	29.8	19.3	6	60
		Weight	13.4	5.5	6.7	23.5
		Height	89.7	17.7	64	118
C	Male (n = 12)	Age	32.2	19.6	4	66
		Weight	13.7	3.9	7	18.5
		Height	90.5	15.5	62	112
	Female (n = 8)	Age	36.2	21.1	2	63
		Weight	15.2	4.6	10.1	23.5
		Height	96.2	11.3	80	110

Age (months); weight (kg); height (cm).
s.d., standard deviation; min., minimum value; max., maximum value.

Two blood samples were taken from each of the children in order to evaluate the secretion of melatonin and β-endorphins, at 9 AM and 9 PM. We also performed a complete haemogram and a study of cellular immunity and recorded total and specific levels of IgE against alimentary and environmental allergens, in the sample taken at 9 AM.

In every case, a somatometric evaluation was obtained, and a questionnaire completed on the personal and family background. The latter information was both general and specific with regard to different kinds of allergic complaints.

Plasma β-endorphins and melatonin levels were analysed by radioimmunoassay (RIA). Plasma melatonin levels were measured in duplicate using a commercial RIA kit (WHB, Bromma, Sweden). In the control samples, the intra-assay 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 melatonin levels were measured in duplicate using a commercial antibody (Stockgrand Ltd, Guildford, Surrey, UK) and ^{125}I-melatonin as a tracer (DuPont NEN, Boston, MA). The protocol used in melatonin RIA was that provided by the antibody manufacturer. The intra-assay and inter-assay coefficients of variation for melatonin RIA were 9.6% and

Table 2. Mean and standard deviation for the haematological and immunological parameters considered in each one of the groups

Variable	Group A (X ± s.d.)	Group B (X ± s.d.)	Group C (X ± s.d.)
Total leucocytes*	9768 ± 2543	10,437 ± 4569	9355 ± 2501
Haemoglobin†	12.173 ± 1.22	12.71 ± 1.35	12.49 ± 0.876
Thrombocytes*	357,067 ± 86,399	256,150 ± 54,449	247,000 ± 64,343
Neutrophils‡	44.44 ± 16.12	31.9 ± 11.60	36.33 ± 10.91
Monocytes‡	5.89 ± 1.84	5.37 ± 1.99	6.92 ± 2.93
Eosinophils‡	2.85 ± 2.57	4.155 ± 3.33	5.405 ± 4.59
Basophils‡	0.58 ± 0.17	0.52 ± 0.52	0.68 ± 0.49
Luc‡	1.52 ± 1.25	1.59 ± 1.84	2.25 ± 1.71
Total lymphocytes*	4287 ± 680	5021 ± 2126	5158 ± 2060
T lymphocytes (CD2)*	3937 ± 882	3261 ± 1295	3694 ± 1416
B lymphocytes (CD19)*	510 ± 132	943 ± 836	1116 ± 565
Helper lymphocytes (CD4)*	961 ± 419	189 ± 935	2140 ± 801
Lymphocytes T8 (CD8)*	918 ± 412	0961 ± 477	947 ± 407
NK lymphocytes (CD56)*	433 ± 182	213 ± 112	193 ± 120
T4/T8 index	1.28 ± 0.18	2.15 ± 0.79	2.68 ± 1.11
Total IgE§	26.28 ± 11.85	239.38 ± 374.42	529.84 ± 866.86

Units: *10^3 cells × μl; †g/dl; ‡percentage; §IU/ml (international units/ml);
Values are expressed as median ± standard deviation (X ± s.d.).

13.2%, respectively. The recovery of added melatonin was 87% and the sensitivity of the assay was 2.5 pg/ml.

Immunoglobulin E was evaluated according to the STRATUS (fluorometric enzyme immunoanalysis) method, which enables IgE to be analysed with a sensitivity of 2–100 IU/ml. The first result was obtained after 8 min, and the rest were obtained at 1-min intervals.

Lymphocyte populations were quantified by means of a haematological analyser, which provided values for leucocytes, lymphocytes, neutrophils and monocytes in the peripheral blood. Cytofluorimetry (Coulter, Excel, Hialeah, FL) was used to evaluate the following lymphocyte subpopulations: B, T, CD2, CD4, CD8 and cells with natural killer activity, with the following monoclonal antibodies, respectively, being used for identification: B4, CD11, CD4, CD8 and CD56.

Statistical analysis of the melatonin and β-endorphin was performed by a two-way analysis of variance using group (A, B, C) and time of sample (9 AM, 9 PM) as the two factors, being as the distribution of both melatonin and β-endorphin variables fit criteria of normality (Shapiro and Willis test). Bonferroni test was used for *post hoc* comparisons. SPSS for Windows, version 14 (SPSS Inc., 1989–2005, Chicago, IL, USA) was used for data entry and statistical analysis.

Results

Table 2 shows the median and standard deviation of the variables and groups evaluated.

Variance analysis was applied to the results obtained to distinguish between the mean values of the three different populations, i.e. the healthy children, those suffering an attack of AD and those suffering the disease but at present in a stable phase, with no symptoms. For the variables with $p < 0.05$ the differences between the mean values were established by applying the Bonferroni test and taking into account, among other aspects, the unequal sizes of the groups. The most significant of the main variables in the study are described below.

Melatonin

The measurement of the levels of *N*-acetyl-5-metoxy-tryptamine (Fig. 1) is one of the most interesting aspects of this study. ANOVA analysis showed significant changes for melatonin as function of both factors, with *F*-value of 15.42 ($p < 0.001$) for comparisons between 'groups' and 56.52 ($p < 0.001$) for 'time of sample' factor. The greater melatonin values was observed in group A (control): 30.46 ± 7.32; 43.53 ± 11.98 pg/ml; at 09:00 and 21:00 hours, respectively), and the lowest in group B (11.68 ± 7.62 and 34.45 ± 15.958 at 09:00 and 21:00 hours, respectively), with significant differences (Bonferroni test) between group A vs. group B ($p < 0.001$) and between B and C groups ($p < 0.001$) although of a smaller magnitude. No significant differences were observed between A and C groups.

β-Endorphin

β-Endorphin seem to be the peripheral effectors of melatonin. ANOVA analysis revealed for

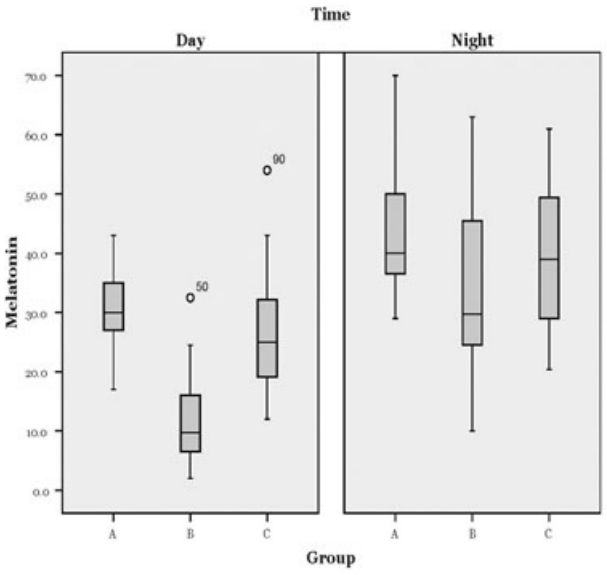

Fig. 1. Day/night oscillation (paired data) of melatonin levels among the three study groups.

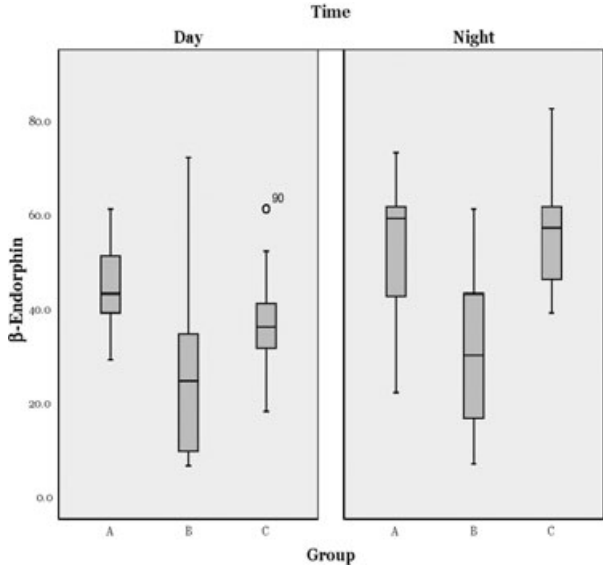

Fig. 2. Day/night oscillation (paired data) of β-endorphins levels among the three study groups.

β-endorphin similar behaviour to melatonin, with statistically significant differences for both factors, with an *F*-value of 21,993 ($p < 0.001$) and 14,146 ($p < 0.001$) for 'groups' and 'time of sample' factors, respectively.

The greater β-endorphin values (Fig. 2) was observed in group A (control: 45.33 ± 9.81; 52.0 ± 15.33 pg/ml; at 09:00 and 21:00 hours, respectively), and the lowest in group B (26.24 ± 17.65 and 31.18 ± 17.65 at 09:00 and 21:00 hours, respectively). Bonferroni analysis showed these results to be due, fundamentally, to the lower values among the children suffering from an attack of AD (group B) in relation with the control group (A, $p < 0.001$); and between groups B and C ($p < 0.001$). The values in the control group and in that of the stable phase of AD were very similar.

Discussion

Atopic dermatitis is an inflammatory skin disease which has been the subject of important studies describing its incidence, pathogenesis and treatment (13–16).

Clinical symptoms are varied and change over time; the main signs are pruritis and prurigo, the lichenification of lesions and eczema, but other, less characteristic lesions have also been described, although these are not accepted by all authors. Diagnosis is basically clinical, and the criteria of Hanifin and Rajka (13) are generally taken to be valid.

Psychoneuroimmunology studies have shown that two major neuroendocrine systems exert a physiological neuroimmunomodulatory function, consisting of the pineal gland and the brain opioid system, provided by immunostimulatory and immunosuppressive effects, respectively. Additionally, there is a well-known relation between the immune and the endocrine systems, by which hormonal factors and neurotransmitters affect the immune system in various ways. The most interesting aspects of the present study are melatonin and its peripheral effectors, the β-endorphins, and the possible differences between their levels in healthy children and in those with AD.

Melatonin (*N*-acetyl-5-methoxytryptamine or aMT) has been the object of various studies in recent years, although very little has been published concerning the effects of this neurohormone on subjects of paediatric age (17–19). The major source of the hormone is the pineal gland and its biosynthesis is regulated by sympathetic nervous signals (noradrenaline) that generate a circadian rhythm synchronized with the hours of daylight (20). The peak production of melatonin occurs during the hours of darkness, with acrophase at 3 AM, while daylight periods are associated with lower levels of melatonin production. As a whole, melatonin is considered to contribute to regulating the sleep–waking cycle and other biological rhythms, gonadal development, and to play an antioxidant, oncostatic, antiageing and immunemodulator role (21). Physiologically, the nocturnal melatonin peak has been associated with a high IFN-γ/IL-10 ratio, i.e. the melatonin rhythm correlated with the rhythmicity in the Th1/Th2

ratio (22); moreover, pinealectomy inhibits IL-2 production and natural killer cell activity (23). Early nocturnal sleep induces a shift in the Th1/Th2 cytokine balance towards increased Th1 activity, which is replaced by a more pronounced Th2 dominance during late sleep (24).

T-helper cells express G-protein-coupled cell membrane melatonin receptors and melatonin nuclear receptors (25), by which the hormone enhances the release of T-helper cell type 1 (Th1) cytokines, γ-interferon (γ-IFN), IL-2, IL-6 and IL-12 (26, 27) production by two human lymphocytic and monocytic cell lines (12). Additionally, human lymphocyte-synthesized melatonin is involved in the regulation of the IL-2/IL-2 receptor system (28), supporting the notion that endogenous melatonin synthesized by human T cells may contribute to the regulation of its own IL-2 production (29) and the possibility that the biological activity of IL-2 may be amplified by pineal indoles. Furthermore, the blockade of the brain's opioid system by a long-acting opioid antagonist, naltrexone, may improve the anticancer effects of IL-2 in humans (30).

Both resting and stimulated human lymphocytes synthesize and release large amounts of melatonin, several times greater than the nocturnal physiological levels in human serum (31). Blocking the lymphocyte machinery required for its biosynthesis produces a significant reduction in melatonin release, and a decrease in IL-2 production, which is restored by adding exogenous melatonin (32). Consequently, in addition to pineal gland-derived melatonin, human lymphoid cells may be an important physiological source of melatonin. That source of this hormone could also be involved in the regulation of the human immune system, possibly by acting as an intracrine, autocrine and/or paracrine substance (31). Melatonin appears to regulate the proliferative and maturational stages of virtually all haemopoietic and immune cell lineages involved in host defence, not only NK cells, but also T and B lymphocytes, granulocytes and monocytes (33). The participation of melatonin in the pathogenesis of AD may be better determined by measuring its concentration in the supernatants of lymphocyte cultures from different groups of patients.

Additionally, the anti-inflammatory properties of melatonin and its metabolites (kynuramines) have been demonstrated in experimental models (34–36).

Given the possibility that melatonin might act by neuroendocrine mechanisms that are different from those commonly accepted, we took into consideration the endogenous opioid system (EOS). The reason for this was partly that melatonin may have analgesic and anticonvulsant effects (36–38), and also because EOS is recognized to play an important, although as yet insufficiently understood, role as an immunoregulator (39). Moreover, a relation between melatonin and EOS in humans has been established (40, 41). Experiments with mice have demonstrated the activation of the EOS system by melatonin (42). These findings complement our own results, as shown by the high degree of correlation between aMT and β-endorphins. The relevance of this relation is highlighted by our study of what happens during an attack of AD. At this point, it is useful to note the role of melatonin and endorphins in the body's response to stress (17, 40, 43, 44). It has been suggested that the latter have negative effects on an organism that is dependent on an exhausted EOS (45). Another study carried out by our group evaluated the aMT–β-endorphin ratio before and after a hyposensitizing protocol. The conclusion was that immunotherapy should incorporate mechanisms that are capable of altering not only the response, but also the level of relations between these two peripheral mediators (46).

As melatonin seems to act via the EOS, in theory it should be possible to reproduce its effects with known opiatergic peptides. To investigate this, the pharmacological effects of aMT have been compared with those of certain endorphins (47, 48). On the basis of the findings, two hypotheses have been proposed that: (i) melatonin may stimulate the production of opiatergic agonists by activated immunocompetent cells; (ii) melatonin may influence the expression or the binding affinity of opiatergic receptors, in an analogous way to its reported action on oestrogen receptors (49). The former hypothesis seems more likely to be correct and, in fact, it has been corroborated that aMT may induce the secretion of opiatergic receptors by immunocompetent cells.

It is still not possible to give a precise description of the mechanism that relates cutaneous manifestations of AD with the type of neuroendocrine response found. Nevertheless, the following may be of interest: during a cutaneous crisis, a certain degree of stress is provoked, and the neuroendocrine response is different from what would be expected under normal circumstances, and so the normal relationship between aMT and β-endorphins, in healthy children, is altered. Taking the above into account, and after analysing previously published findings, it is evident that the pineal gland participates in the regulation

of the immune response via the rhythmic, circadian production of aMT, which in turn possesses immunomodulating and antistress properties. It seems reasonable to suggest that melatonin works through a common mechanism, either at the central level on the neuroendocrine system, or peripherally on the immune system, to optimize the homeostatic efficiency of the neuroendocrine network.

With regard to the rhythmic hormonal secretion in patients with AD, studies have shown that in certain isolated cases the circadian production of melatonin is lost (50). In this study, on the basis of only two samples from each patient (this limitation being imposed by ethical reasons), we were unable to determine whether the circadian rhythms of melatonin and/or β-endorphin are altered. Nevertheless, the present study reveals that during an attack of AD (group B), there occurs a diminution in aMT, which is more marked during daylight hours. On the other hand, when the crisis has passed, the tendency is towards normalization, such that near-normal values are found among the subjects in group C. These data coincide with the findings of Schwarz et al. (50), who described a lower average level of circulating melatonin in individuals with AD, although the latter study was based on patients who were not of paediatric age.

To explain the above-described fall in levels of circulating melatonin in individuals with AD, we should also take into account the neurobiochemical mechanism that the cell implements to produce melatonin. During the daylight hours, conduction through the retina-suprachiasmatic nucleus-superior cervical ganglion-pinealocyte pathway is inhibited, and the circulating levels probably arise from an extrapineal source (51). Moreover, it has been shown that the number of β-receptors in the pinealocyte membrane also falls dramatically. During AD there is a marked diminution in the numbers of these receptors (52), which could partially explain the lower levels of melatonin found during the night. This, then, might be interpreted as a dysfunction of the central nervous system, culminating in a disorder of the vegetative nervous system, with the associated dermatological manifestations.

Another aspect of undeniable interest is the highly changeable psychic character shown by children with AD, which has led to the formulation of the so-called 'indole-aminic theory' of affective disorders, based on reduced levels of 5-hydroxytryptamine (a direct precursor of aMT). This fact has been observed both among those suffering affective disorders and among those with AD, as in both groups of patients, irrespective of other conditions, there exists an evident link between psychic changeability, the indole-aminic theory and reduced levels of melatonin. Biological hypersensitivity to environmental stimuli is a fundamental feature of atopy. There is growing evidence that psychological stress, as a social pollutant, disrupts inflammation-related biological systems, which modulates the hypersensitivity response. Once again, the Th1–Th2 balance may be crucial to the interpretation of quantitative differences in cytokine expression in response to environmental stimuli-like stress, which may overlap with those altered by physical pollutants and toxicants (53). Reinforcing the possible clinical utility of melatonin, in one experimental model of stress-induced skin disorders, chronic melatonin treatment reduced the infiltration and activation of mast cells in the dermis (54). The reduced daytime circulating levels of melatonin in AD, as shown by the data presented in our paper, may reflect the reduced production of melatonin of extrapineal (immunological) origin. If other studies confirm our data, this deficiency may be restored by melatonin treatment.

The above-described information led us to consider the potential utility of melatonin in the treatment of AD, in part due to the sedative and general stabilizing properties of the hormone and also because it may help restore the Th1/Th2 balance (27) for adequate immune system homeostasis. However, the correct therapeutic use of melatonin should be based on a complete understanding of its action mechanism. Melatonin may also act on T-helper type 2 cells (Th2); a binding site for ^{125}I-melatonin on these lymphocytes in the bone marrow has been identified (55). Another point advising caution is that the hormone may exert a proinflammatory action. The nocturnal plasma concentration of melatonin in rheumatoid arthritis patients is higher than in healthy controls, and their synovial macrophages express a specific binding site for melatonin with an increased production of IL-12 and nitric oxide and may exert a disease-promoting role in rheumatoid arthritis (32).

Schwarz et al. (50) remarked, furthermore, on the fact that when individuals suffering AD were treated with melatonin, the peripheral profile of aMT was virtually unaffected.

All these considerations lead us to conclude that within AD, the primary pathogenic nucleus probably comprises a central neuroendocrine disorder. Further studies are required, oriented fundamentally towards neurovegetative and hormonal aspects, for better comprehension of the process.

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