

Melatonin and β -Endorphin Changes in Children Sensitized to Olive and Grass Pollen after Treatment with Specific Immunotherapy

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Key Words

Melatonin · β -Endorphin · Immunotherapy, specific · Grass pollen · Olive pollen · Allergy, respiratory · Children

Abstract

Background: Specific immunotherapy for respiratory allergy, a seasonal disease, significantly reduces the inflammatory process, attenuating the clinical symptoms. The mechanism for the clinical beneficial effect of immunotherapy has not yet been clarified. Melatonin shows a circadian and seasonal variation and together with the endogenous opioid system plays an immunomodulatory role acting on both specific and nonspecific immunity responses. Thus, the possibility that immunotherapy involved changes in the melatonin-opioid system was investigated. **Methods:** Thirty-five children aged 3–15 years with rhinitis and asthma due to olive + grass pollen sensitization were studied. The patients were treated with depot extracts containing the identified allergens with increasing doses from 1 to 1,000 IU/ml during 3 months. Melatonin, β -endorphin, total and specific IgE and IgG4 were determined before and after treatment. **Results:** All children showed a significant improvement of their symptoms at the end of the treatment, coin-

ciding with a significant drop of both melatonin and β -endorphin levels. Total IgE decreased in most of the cases although the mean values did not show significant changes. Specific IgE and IgG4 were also unchanged. A significant correlation between melatonin and β -endorphin and between β -endorphin and IgG4 was found before immunotherapy, and these correlations disappeared when the treatment was finished. **Conclusions:** The decrease in the levels of melatonin and β -endorphin suggests the disappearance of their stimulating influence on the immune system. Thus, hyposensitization after immunotherapy may involve, at least in part, the inhibition of the immunoenhancing properties of the melatonin-opioid system.

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Introduction

In the current pediatric context, respiratory allergy presents one of the most important social problems because of its clinical, therapeutic, educational and health implications [1]. During the last years, several changes have been made in their treatment and prognosis. The use of β_2 -adrenergic stimulants, aerosol therapy and topic anti-inflammatory drugs have drastically changed the

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clinical situation of respiratory allergy [2, 3]. Anyway, mainly during certain seasons of the year there is an increase of the frequency of bronchospasms with repeated crises [1]. When the allergen is identified, it is easy to apply a specific immunotherapy, i.e. the administration of increasing doses of the allergen to get a status of immunological tolerance [4, 5]. Immunotherapy provides a mechanism to reduce the inflammatory status, attenuating the symptoms. In spite of the efficacy of this treatment, the mechanism(s) by which the clinical beneficial effect is reached after this therapeutic approach is not known.

Melatonin, a biogenic amine and neuromodulator, shows a circadian variation in plasma due to its rhythmic production by the pineal gland [6]. This hormone is regulated by the photoperiod and exhibits a circannual variation. Melatonin is produced by several other tissues of the body, including the immune system [7]. Recently, it has been found that the key enzymes of melatonin production, N-acetyltransferase and hydroxyindol-O-methyltransferase, are present in most of the organs of the body, including spleen, thymus and platelets [8]. These data suggest that these cells may produce melatonin if the enzymes are adequately induced, and explain its production by cells of the immune system.

Classical actions of melatonin induce effects on pubertal development [9, 10] and sleep [11]. Other studies have identified melatonin as an antistress [12, 13], anticonvulsant [14, 15] and antiexcitotoxic [16–18] compound. The antioxidant properties of melatonin [19–21] are now recognized to be the basis for some of its effects, including its antistress property.

The antistress action of melatonin is closely related to its immunoenhancing properties [22–26]. Melatonin has both direct and indirect actions on the immune system [27], and it increases the specific and nonspecific immunity [28, 29]. Melatonin is known to activate T helper cells by means of direct binding to melatonin receptors on both Th1 and Th2 cells [25, 30, 31]. Some of these effects of melatonin are produced by increasing cytokine production [28]. Besides, a relationship between melatonin and the endogenous opioids is now recognized, constituting a neuroendocrine axis regulating the immune system [32]. Moreover, melatonin increases the antibody response by a mechanism involving the endogenous opioid system [33], and thus, the existence of a neuroimmunoendocrine axis involving melatonin, endogenous opioid peptides and the immune system has been proposed [30, 32, 34].

Although the clinical efficacy of the immunotherapy with allergens in the treatment of the respiratory allergic

diseases (rhinitis and asthma) is currently demonstrated [35], the mechanisms involved in its clinical efficacy are not yet known. Thus, we consider it worthwhile to examine whether the hyposensitizing immunotherapy in children sensitive to olive and grass pollen modify the response of the immunoendocrine-opioid system in terms of immunoglobulin, melatonin and β -endorphin levels.

Materials and Methods

Patients

A total of 35 patients (8 girls and 27 boys), aged between 3 and 15 years, were studied at the Granada University Hospital. Informed consent was obtained in all cases from the parents and from the hospital's Ethical Committee of the Granada University Hospital, according to the 1983 revised Helsinki Declaration of 1975. The clinical history and the somatometric and analytical data were recorded. The inclusion of the patients in the study was done according to the following criteria: (1) previous clinical history of rhinitis and/or asthma during the pollen season; (2) positive cutaneous test to grass and olive pollens, and (3) total IgE >0.1 IU/ml. Patients with pathologies requiring a treatment contraindicated when using immunotherapy [30] were excluded from the study.

Treatment and Analytical Methods

The patients were treated with standardized extracts of the main allergen identified (ALK-ABELLÓ, Pangramin® Depot). The treatment was given before the season of the year when the allergy began. The extracts (biologically standardized depot extracts) were initially administered at doses of 1 IU/ml and gradually increased up to 1,000 IU/ml until the end of the treatment. The patients received two subcutaneous injections per week during 3 months. To be efficient, the treatment with immunotherapy should be started before the onset of the allergy, which in our case occurs in spring. For this reason, all children were studied between November and January. With this schedule we avoid melatonin circannual variation influencing our results. As a result of ethical considerations, there was no control group with untreated children with grass/olive allergy, since the children were selected for this study because they did not respond to other types of therapy. Besides, this study was designed to evaluate the effects of the 3-month immunotherapy treatment and thus, each patient was his own control. Anyway, a group of children not suffering from allergy was studied during a similar period of time to assess the levels of the measured parameters in a normal population of a sex- and age-matched group (control group).

Blood samples were taken between 9:00 and 10:00 h before and after treatment. Blood was centrifuged at 3,000 g for 10 min, and serum was separated and frozen at -20°C until the assays were done. Melatonin was measured by a commercial radioimmunoassay (DLD Hamburg, Germany) and a quality control was performed showing an intra- and interassay coefficients of variation of 11.3 and 6.3%, respectively. The recovery of added melatonin was 84.4% and the sensitivity of the assay was 5 pg/ml [36]. The concentration of β -endorphin was determined by a commercial radioimmunoassay kit (Nuclear Corporation, Stillwater, Minn.) and expressed in pg/ml. Total IgE, specific IgE and allergen-specific IgG4 against olive + grass pollen were determined by fluorometric enzyme-immunoassay tech-

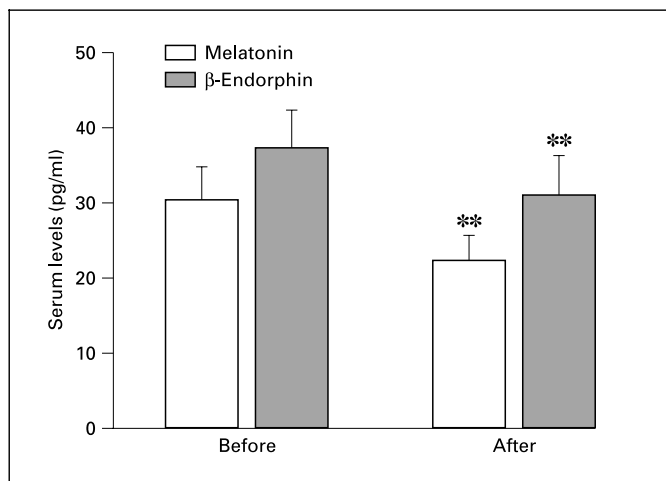


Fig. 1. Comparison of the serum concentrations of melatonin and β -endorphin before and after immunotherapy treatment (Student's t test). ** $p < 0.001$ vs. before treatment.

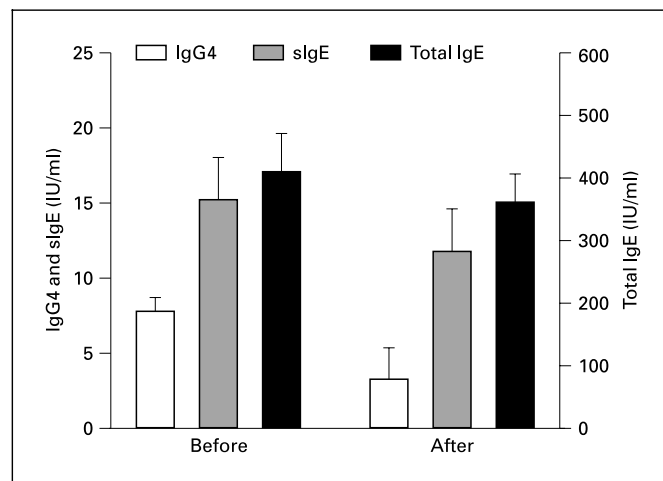


Fig. 2. Comparison of the serum levels of IgG4 (sIgE) and total IgE before and after immunotherapy treatment (Student's t test).

niques using a Stratus equipment for total IgE, or by IgE FAST-Plus and IgG4 FAST-Test kits for specific IgE and IgG4, respectively. Values for immunoglobulins are expressed in IU/ml.

Statistics

Data are expressed as the means $\pm$ SEM. Comparison tests for paired data (Student's t test), correlation and regression analyses, and the Fisher 'z' transformation test were used.

Results

All children showed a significant improvement of their symptoms at the end of the immunotherapy. Figure 1 shows the changes in melatonin and β -endorphin before and after the treatment. Melatonin and β -endorphin significantly decreased with treatment ($p < 0.001$), suggesting an inhibitory effect of immunotherapy on these variables. The levels of melatonin (32.6 ± 3.2 pg/ml) and β -endorphin (36.4 ± 3.3 pg/ml) in the control group were similar to those of the group with allergy before treatment (30.3 ± 3.6 and 37.2 ± 4.7 pg/ml). After treatment, the levels of melatonin (30.1 ± 4.4 pg/ml) and β -endorphin (36.8 ± 4.2 pg/ml) in the control group were unchanged, whereas melatonin (22.4 ± 3.1 pg/ml) and β -endorphin (31.2 ± 4.2 pg/ml) levels decreased significantly in the allergy-treated group.

Figure 2 shows the levels of IgG4 (7.8 ± 0.9 and 3.3 ± 2.1 IU/ml), specific IgE (sIgE) (15.2 ± 2.8 and 11.8 ± 2.8 IU/ml) and total IgE (408.4 ± 61.4 and 363.7 ± 44.1

IU/ml) before and after treatment, respectively. Although there was a decrease in the levels of total IgE in all children considered individually, the mean value after treatment did not reach significance. Also, specific IgE and IgG4 did not change significantly. The levels of the immunoglobulins measured in the control group did not show significant differences with their levels in the allergy group (data not shown).

The second analysis consisted of determining the relationship existing between each variable before and after the immunotherapy. A significant correlation was found between the melatonin values before and after treatment ($r = 0.51$, $p < 0.01$) and between β -endorphin levels before and after immunotherapy ($r = 0.70$, $p < 0.001$). IgE also showed a significant correlation between its values before and after treatment ($r = 0.62$, $p < 0.001$). However, no correlations were found for specific IgE and IgG4.

We next examined the existence of a relationship between the measured variables and the influence of immunotherapy on it. A significant correlation between melatonin and β -endorphin levels before treatment ($p < 0.001$) was found, which disappeared after the administration of immunotherapy (table 1). IgG4 and β -endorphin were also significantly correlated before treatment ($p < 0.05$), and the treatment also disrupted this relationship. No correlation between total IgE and specific IgE and melatonin or β -endorphin was found during the study.

Table 1. Correlation between the levels of melatonin, β -endorphin and IgG4 before and after the immunotherapy

Variables	Before treatment		After treatment	
	r	p	r	p
Melatonin/ β -endorphin	0.77	**	0.31	n.s.
Melatonin/IgG4	0.43	n.s.	0.17	n.s.
β -Endorphin/IgG4	0.56	*	0.33	n.s.

* $p < 0.05$; ** $p < 0.001$. n.s. = Not significant.

Discussion

Our results show two important findings, i.e. melatonin and β -endorphin significantly decreased after immunotherapy, and the correlation between these variables and between β -endorphin and IgG4 disappeared with the treatment. These results suggest that the allergen immunotherapy used not only alters the immune response of the children with respiratory allergy [3–5] but also alters the relationships between the components of the endocrine, opioid and immune system studied here.

The main characteristics of the inflammatory allergy in humans is the IgE-dependent basophilia and activation of mastocytes, and the cytokine-dependent eosinophilia. After their activation, the Th1 cells produce IFN- γ and IL-2, and the Th2 cells produce IL-4, IL-13 and IL-5. Both cells also produced IL-3 and colony-stimulating factors. IL-4 is important for the increase of IgE, a process inhibited by IFN- γ , which in turn may be induced by IL-12 [37, 38].

A series of experiments suggest a relationship between the immune system, melatonin, and the endogenous opioid system [27, 28]. Certain opioids are involved in the action of melatonin on the immune system [22, 25, 33], and melatonin exerts some of its effects through μ -opioid receptors [39]. The immunoenhancing and antistress properties of melatonin are closely related effects, and both of these effects may be blocked by administration of the opioid antagonists naloxone or naltrexone, whereas dynorphin and β -endorphin reproduce the immunological effects of melatonin [23]. A direct effect of melatonin on the immune system was further demonstrated in several experimental paradigms [40, 41]. Th1 and Th2 lymphocytes are targets for melatonin action [25, 28, 40], and the indoleamine enhances the release of Th1 cytokines, such as IFN- γ and IL-2 as well as of novel opioid cytokines which cross-react immunologically with both IL-4

and dynorphin B. This IL-4-like substance may stimulate adherent stromal cells to produce granulocyte/macrophage-colony-stimulating factor [42]. Melatonin also enhances the production of IL-1, IL-6 and IL-12, this last cytokine only in the presence of IL-2, in human monocytes [43]. Although there are few reports concerning the effects of immune products on the pineal production of melatonin, IFN- γ seems to stimulate the production of melatonin and other indoleamines in human T lymphocytes and macrophages. Besides, IFN- γ and colony-stimulating factors modulate the melatonin production in cultured rat pineal gland.

At least part of the clinical efficacy of immunotherapy depends on the inhibition of the Th1 and Th2 cell responses [38, 44]. The immunotherapy reduces the seasonal increase in specific IgE, whereas high levels of IgG4 are associated with the failure of immunotherapy. After Th2 inhibition, IL-4 decreases, explaining the reduction in sIgE after immunotherapy. On the other hand, although in some types of immunotherapy an increase in IFN- γ was reported, recent data showed an inverse correlation between IFN- γ and duration of allergen immunotherapy [44]. Thus, the suppression of Th1 cells may reduce IFN- γ , opioid cytokines and colony-stimulating factors, which may be responsible for the immunotherapy-dependent decrease of melatonin levels. These changes may be also responsible for the disruption of the melatonin/ β -endorphin correlation found before treatment, and the subsequent β -endorphin decrease.

Taking together, these pieces of evidence support an immunoenhancing property of melatonin-opioid axis. In our study, we had a hyperreactive status with an increased immune response. This situation reverted after hyposensitizing immunotherapy that reduced the specific immune response. The decrease in the inflammatory response parallels the decrease in the melatonin and β -endorphin levels, thus reducing the potential immunoenhancing effect of the melatonin-opioid axis. The melatonin-opioid target seems to be antigen-activated T lymphocytes [28, 45, 46], which displays naltrexone-sensitive opioid receptors. This may explain the parallelism found between melatonin and β -endorphin, and the loss of their relationships after immunotherapy. It is reasonable to suppose that the dissociation of the melatonin-opioid axis due to the treatment reduced the effectiveness of melatonin in stimulating the immune system including the antibody production [33]. These results also suggest that, besides the influence of the melatonin-opioid axis the immune system, an inverse relationship, i.e. a feedback effect of the immune system on the melatonin-opioid axis, may exist.

Other mechanisms have been proposed to explain the relationship between the immune system and melatonin-opioid axis. Melatonin treatment affects the reproductive capacity, the production of sex hormones, and certain immune functions [47, 48], suggesting the involvement of the hypothalamus-pituitary axis in these responses. However, in our study we did not find significant differences between the responses to treatment in girls and boys, discarding a sex-dependent effect of the immunotherapy. On the other hand, melatonin antagonizes the suppressor effect of corticoids on the immune system [49], and the existence of a pineal-adrenal axis has been suggested [50]. The photoperiod transducer melatonin synchronizes the organism with the photoperiod [6]. Cytokines exhibit a diurnal rhythmicity and besides cortisol, melatonin also entrains their rhythm. The antibody production is also modulated by the length of the days and dependent upon the pineal [51]. Thus, the influence of the season and photoperiod on melatonin production and on the immune function [52] may indicate changes in the sensitivity to allergens in the different seasons of the year. This suggests that the nature of the immune response, for example to allergen, may be modified by the time of day when antigen is administrated [53].

In summary, the phenomena of immunotolerance and reduction of the immune response achieved with hypo-sensitizing therapy lead to a decrease of both melatonin and β -endorphin levels, and disrupted their correlation and that of β -endorphin with IgG4. Thus, there is a new neuroendocrine argument justifying immunotherapy. Melatonin may be part of an integrative system coordinating immunological processes. Direct and indirect interactions between the melatonin-endogenous opioid system and the immune system exist, and it appears that melatonin may play a complex physiological role in neuroimmunomodulation. For these mechanisms melatonin may counteract immunodeficiency when the immune system is downregulated. In turn, when the immune response is exacerbated, melatonin and the endogenous opioids should decrease [43], as in our study after immunotherapy.

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