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REVIEW ARTICLE

Melatonin in immunity: comparative aspects

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Abstract

Pineal gland, by the diurnal rhythm of synthesis and release of its principal hormone, melatonin (MEL), is involved in reciprocal relationships between neuroendocrine and immune systems, responsible for keeping internal homeostasis in vertebrate animals. In this paper the experimental data, indicating that both strategic (developmental, thus antigen independent) and emergency (evoked by antigenic activation of the mature immune system) levels of interactions between pineal gland and immune system, operate in mammals and birds, are reviewed. The cells and organs of immune system using membrane receptors as well as nuclear orphan receptors perceive MEL message. Effects exerted by MEL on immune parameters are different, and depend on several factors, including dose and way of MEL application, species, sex, age of animal, its immune system maturation, way of immune system activation, and parameter examined, as well as the season, circadian rhythm of both immunity and pineal gland function, stressful conditions, accompanying experimental procedure, etc. In turn, lymphoid organ-derived hormones and cytokines, soluble factors secreted by activated immune cells act as messages understood by the pineal gland, closing the regulatory loop of the bi-directional functional connections between both systems.

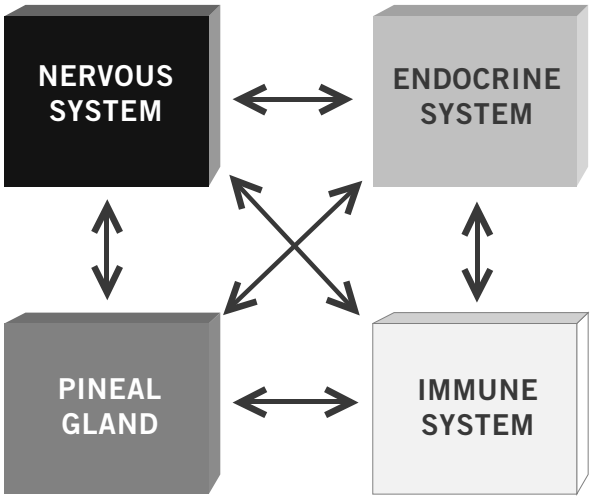

Fig. 1. Pineal gland as a part of the regulatory network involved in homeostasis keeping system.

Introduction

Maintaining of homeostasis within the vertebrate' body is possible due to a strict cooperation between the neuroendocrine and immune systems. Both systems take important information from other sources (external environment and antigens, internal milieu, cognitive and non-cognitive stimuli, physical and psychical stress), and communicate intensively. In the healthy organism, there is a profound modulation of immune reactivity by neurotransmitters and hormones and conversely, immune cells-derived soluble mediators, cytokines, have an effect on neuroendocrine function. It means that the communication between neuroendocrine and immune systems includes the use of common signal and recognition molecules [1].

In the last two decades, the role of melatonin (MEL), the main neurohormone synthesized and released by the pineal gland, as a neuro-modulator has been examined extensively [2] and its participation in the immunomodulation has been accepted [3]. Due to its particular ability to transduce an external information on light, not perceived by both neuro- and endocrine systems, into a biochemical message understood by the whole body, pineal gland may be considered as a separate part of the homeostasis keeping system (Fig. 1). This message, consisting of the daily rhythm of MEL synthesis and release, is thereafter transmitted to the immune system using several intermediate mechanisms. These mechanisms will be discussed in the present paper. Moreover, the experimental data indicating that the pineal gland itself is able to receive the information from immune system will be presented as well.

Levels of communication between neuroendocrine and immune systems

Interrelationships between neuroendocrine and immune systems may be considered according to Fabris [4] at two different levels: strategic level and emergency circuit. The first one, an antigen-independent strategic level, is responsible for the normal development and maturation of both immune and neuroendocrine systems. The second, an emergency circuit, takes place during developing immune response. It involves different immune cell-derived soluble mediators, containing not only classical cytokines but also a large number of soluble factors, such as peptides, identified to date in the neuroendocrine system. These immune-derived molecules are the main source of information exchanged between immune and neuroendocrine systems, playing the autocrine, paracrine and possibly endocrine roles in the physiology of the immune and neuroendocrine systems.

Melatonin receptors within immune system

The same levels of interactions between pineal gland and immune system are presented in this review, but first the assumption is necessary to make that the glands and cells of the immune system may understand the MEL message. In fact, the iodoMEL binding sites were detected first in the membrane preparations from spleen of hamster [5] and then in other mammalian species and birds [6-9]. Binding of 2-[^{125}I]-iodomelatonin was specific, stable, saturable, reversible, time-dependent and the binding parameters (K_d and Bmax) fulfilled all criteria of the membrane-bound, high affinity receptors [10]. Subsequently, two classes of membrane-bound MEL (high- and low-affinity) binding sites have been identified in human peripheral blood leukocytes [11, 12] as well as in the lymphoid glands and cells of the different mammalian [13] and avian species [14]. Now, it is well accepted that membrane MEL receptors belong to G-protein-coupled (probably Gi/Go) receptors family, and MEL signal transduction involves cyclic nucleotide pathway [15]. Moreover, as MEL molecule is both water- and lipid soluble and may easily penetrate into the cell [2], the presence of cytosolic and/or nuclear receptors was postulated. In fact, MEL binding to the cytosolic molecule calmodulin was demonstrated in some cell lines [16] and at the nuclear level MEL binding sites belonging to the RZR/ROR orphan receptor family, has been demonstrated [17].

Strategic level of relationships between pineal gland and immune system

Already 20 years ago it was demonstrated that functional pinealectomy, achieved by exposure of mice for some generation to constant illumination causing suppression of MEL synthesis, produced all the symptoms of runting syndrome, with severe impairment of body growth and antibody production, and an atrophy of lymphoid tissues [18]. However, demonstration of the reciprocal dependence during the embryonic development of the pineal gland and immune system in mammals is methodologically difficult, if not impossible, and the effects of the postnatal pinealectomy are strongly dependent on species and time of ontogeny when the surgery was performed. The avian embryo offers an excellent model for this kind of study, especially because the thymus and pineal gland start to develop at the same time. Retarded development of the primary lymphoid gland (thymus and bursa of Fabricius) and a decreased immune response, accompanied by the significant changes in the biogenic amines concentration in the spleen, brain and hypothala-

mus were found in chicken embryo, pinealectomized at 96 h of incubation, therefore developing without any influence of the pineal gland [19]. These results clearly indicate the necessity of an intact pineal gland for the normal development of immune system, and suggest that the influence of the pineal gland may be exerted either directly on the lymphoid gland, or/and indirectly, via neuroendocrine network.

Existence of the strategic relationship between immune system and the pineal gland was also demonstrated in experiments involving bursa of Fabricius, a primary lymphoid gland existing exclusively in avian species, and responsible for the maturation of B-cells. Historically, the discovery in 1956 by Bruce Glick from Ohio State University, that the avian cloacal gland, bursa of Fabricius, is a lymphoid gland that histologically resembles the thymus [20], has created a background for understanding the development and function of the humoral immune response in vertebrates. In the bursal microenvironment, composed with the different type of epithelial cells and soluble factors, B-cell precursors undergo the development and maturation to be able thereafter to synthesize and secrete immunogobulins at the periphery [21]. Among the soluble factors of the bursal microenvironment, a low-molecular tripeptide LYS-HIS-GLY-NH_2 was recognized as a bursal hormone, named bursopoietin or bursin [22], that induces the development of B-cells from their avian and mammalian precursor *in vitro*. Subsequently, it was demonstrated that early embryonic bursectomy not only diminished chicken immune response but also influenced the circadian rhythm of the pineal gland function expressed as a decreased nocturnal peak of the pineal NAT activity and serum MEL level [23]. On the other hand, early posthatch chicken pinealectomy abolished the circadian rhythm of non-specific immune parameters, restored by prolonged treatment with very low, physiological doses of MEL [24].

During the postnatal life of mice either surgical pinealectomy [25] or a pharmacological inhibition of pineal gland function [26] caused an involution of the thymus, associated with a depression of cell-mediated immune response and significantly reduced antibody production, NK cell activity and lymphokine production [27]. Evening administration of MEL restored the normal immune response. In mice with an intact pineal gland MEL treatment enhanced production of antibodies against T-dependent antigens, and counteracted, by a reconstitution of the thymus structure and function, the immunosuppression induced by stress, corticosteroids [28] and age-associated decrease in immune functions [29]. Recently, a parallel pattern of the diurnal rhythms of MEL and thymic hormones thymosin α_1 and thymulin was demonstrated in rats and humans [30]. In rats, continuous light and pinealectomy caused a decrease in the content of both hormones in the thymus and their concentrations in serum, whereas MEL injection increased both hormones content or concentration, and stimulated the prothymosin α gene expression. This is in agreement with previous finding that daily MEL injections prevented age-related apoptotic changes in old rat thymus, and that MEL addition into cultured thymocytes caused a decrease in glucocorticoid-induced percentage of apoptotic cells [31].

Taken together, both previous and new results collectively seem to explain, at least partly, the mechanism thereby pinealectomy diminished the T-dependent immune function in mammals, whereas MEL supplementation restored this function. As the thymic hormones in avian species [32, 33] are quite different from those in mammals (in particular, there are no avian thymic peptides structurally similar to the thymosin and thymulin, identified in mammalian thymus) [34, 35], it could be one of the reasons why, in the same experimental schedule, an immunostimulatory and anti-glucocorticoid effects of MEL were observed in mice [26, 28] but not in chickens [36, 37].

Another evidence of the involvement of MEL in the immune system maturation and function is demonstration that this hormone is not only present, but also synthesized within hemopoietic tissue, i.e. in the bone marrow, a main place where the immunocompetent cells are generated [38]. Hematopoietic effect of MEL on the bone marrow macrophages and IL-1 production [39] as well as on the NK and Mo/Ma number in the bone marrow and spleen was also demonstrated [40]. Therefore, it is admissible that MEL present and synthesized in the same place might exert a paracrine effect, especially in immunocompromised animals. Additionally, it was shown [41] that peripheral blood mononuclear leukocytes have a capacity to transform serotonin into MEL, which can act not only locally but also close a regulatory loop between neuroendocrine and immune systems. The summary of the experimental evidences of the strategic interrelationships between pineal gland and immune system is presented in Fig. 2.

Melatonin influence on activated immune system (emergency circuit)

The emergency level of pineal gland-immune system relationships is examined extensively in various experimental approaches, but the results are still controversial. There are several reasons to explain the diversity of the effects exerted by MEL on activated immune system; the effects observed strongly depend on the experimental model, including species, sex and age of the animal examined, the way and extent of immune system activation, immune parameter examined, MEL dose and duration of treatment, as well as on the factors not frequently taken into consideration, such as the circadian rhythm (both the pineal gland and immune system function), season, stressing conditions accompanying the experiment, etc.

Answers for two questions are crucial for understanding the emergency level of relationships between pineal gland/MEL and immune system:

(i) how and by what mechanism(s) MEL influences the different aspects of immune system activity?

(ii) whether and by what mechanism(s) the activation of the immune system is perceived by the pineal gland?

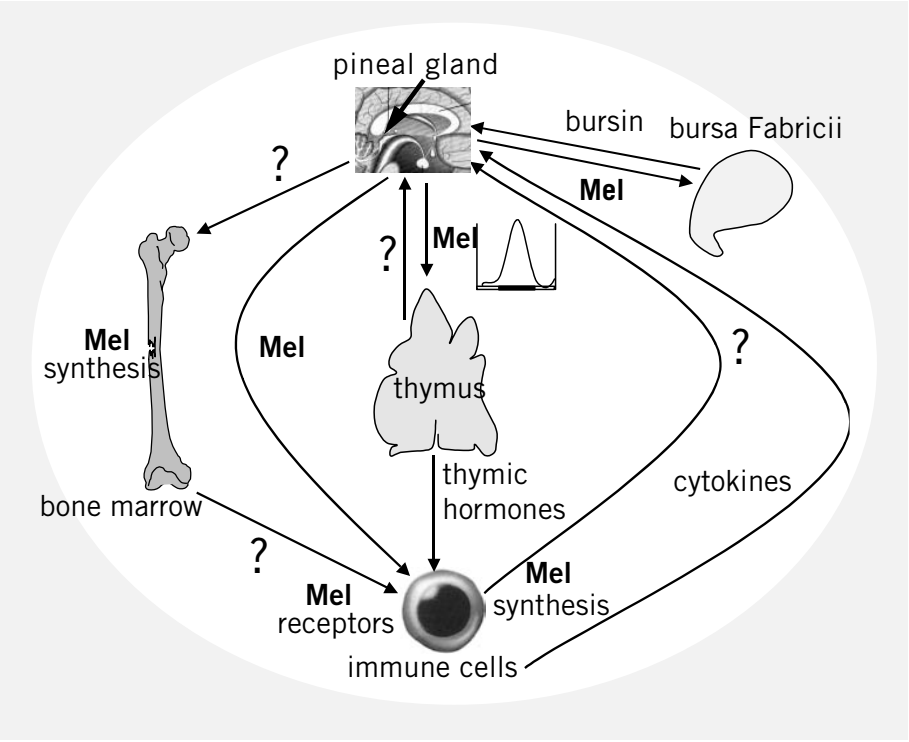

Fig. 2. Involvement of the pineal gland and melatonin in the strategic level of reciprocal communication with immune system; ? indicates pathways supposed but not experimentally proven to date.

In particular, the second question seems to be of basic importance, especially because of the well-established light-driven regulation of MEL synthesis and its circadian and annual rhythms. As far as the first question is concerned, the species-specific MEL effect has to be taken into consideration, especially when the results of experiments performed using different animal species or *in vitro* are applied to the human beings.

MEL has been shown to modulate several immune functions, namely, antibody production, lymphocyte proliferation, ADCC activity, NK cell cytotoxicity, cytokine synthesis and release, etc [for review see 3, 42, 43]. Taking into consideration a versatility of MEL function, even within the immune system [43], its facility to penetrate into cell as well as the presence of binding sites both in the cell membrane and in the nucleus [12, 17], one can assume numerous mechanisms involved in its regulatory activity.

Stimulatory effect on antibody synthesis in mice was found to be mediated by the endogenous opioids (EO) produced under MEL influence by antigen-activated immunocompetent cells [26, 28]. These results were obtained in experiments *in vivo*, in which the stimulatory MEL effect on anti-SRBC antibody production was reversed by naltrexone, an antagonist of the opioid receptors, and this immuno-enhancing and anti-stress effect was mimicked by the exogenous β-endorphin and dynorphin 11-13. Subsequently, the expression of POMC gene, encoding endogenous opioids was demonstrated in rat lymphoid glands [44, 45]. Similarly, treatment of chickens with MEL, although inefficient to stimulate antibody production, caused the POMC and enkefalin genes expression in spleen [Dziwinski, unpublished data]. Therefore, we hypothesized that MEL-induced immuno-opioids most probably act differently in mammals and birds and it may be another reason for the failure to demonstrate the immunostimulatory effect of MEL in chickens [36, 37] using the experimental schedule efficient in mice [26, 28]. We have already preliminary data indicating the different effect of morphine, an agonist of opioid receptors, on inflammatory reaction in various vertebrate species [46].

Diversity of MEL effects within mammalian immune system is briefly summarized in Table 1, using as the examples the contradictory results obtained by different authors in various experimental approaches in relation to two very important immune parameters, i.e. NK cells number and activity, and some cytokine synthesis and secretion by activated immune cells.

Melatonin and seasonal changes in immunity

One of the most interesting relationships between MEL and immune system are season-dependent changes in immunity observed in wild-living animals both in nature and in laboratory conditions, when the animals are kept in different lighting regimes. MEL, transducing photoperiodic information, appears to be the primary hormone involved in the seasonal synchronization of the reproductive function of many vertebrates [2], and it affects also a wide range of seemingly unrelated physiological, morphological and behavioral processes. Many seasonal adaptations, including suppressed breeding, increased thermogenesis, diminished growth, and enhanced immune function have evolved to help animals coping with the annual changes in environmental energy demands [57]. Immune response is energetically expensive and requires the resources that could be allocated to other functions. It is therefore suggested, that individuals "optimize" immune function and allocate energetic resources between the costs of immune function and other maintenance or reproductive functions [58], and maintain the highest energetically possible

Table 1. Diversity of effects exerted by the pineal gland and MEL on NK cells and cytokine synthesis

NK cells	Ref.	Cytokine synthesis	Ref.
Chronic MEL treatment ↑ number and activity	47	Px ↓ and one pharmacological MEL dose restores IL-2 secretion in mice	27
Px ↓ and one pharmacological MEL dose restores NK activity in mice	27	MEL-induced ↑ IFNγ by murine splenocytes	48
MEL-induced ↓ NK activity in vitro in human blood cells	49	MEL recovers age-related ↓ in IL-2 production in mice	50
MEL treatment of healthy volunteers does not influence NK number and activity	51	MEL alone does not induce but inhibits PHA-stimulated production of IFNγ and TNF by human PBL in vitro	52
Long-term MEL administration does not recover the impairment of NK activity in aging mice	53	MEL stimulates or inhibits, in concentration-dependent way, mitogen-stimulated IFNγ synthesis in human PBL	54
One-or two-week feeding with MEL ↑ NK number in bone marrow and spleen of mice	40	MEL ↑ IL-2, IL-6 and IFNγ by human CD4+ blood lymphocytes	17
Acute MEL administration ↑ NK responsiveness to IFN-γ, chronic treatment ↑ spontaneous activity in healthy volunteers	55	Px ↓ and chronic MEL treatment restores IL-2 secretion in mice	56

level of immune function. Nelson and co-workers [57–59] proposed working hypothesis that long-night pattern of MEL enhances immune function in advance of stress-induced immunosuppression. Therefore, in nature short photoperiods provide physiological anticipation of the difficult winter conditions, when the low temperature and limited food availability could compromise immune function. Increased MEL synthesis may function as an indication to direct the energy from growth and reproduction to thermogenesis and immune functions. Winter survival in small animals is hypothesized to require a positive balance between short-day-enhanced immune status (higher MEL level) and stress-induced immunosuppression, because in this season several stressors are involved: overcrowding, increased competition for scarce resources, low temperature, reduced food availability, increased predator pressure or lack of shelters. Furthermore, in a tropical mammal the Indian palm squirrel, *Funambulus pennanti,* MEL acts as an immunoenhancing agent and plays an important role in the synchronization of its immunity with the seasonal changes in environmental conditions and gonadal status [60].

Influence of the immune system activation on the pineal gland function

To close a regulatory loop between immune system and pineal gland function it is absolutely necessary that the messages sent by the activated immune system were understood by the pineal gland, i.e. influenced pineal gland activity and, therefore, MEL synthesis. Scarce evidence for that comes from the studies performed in both mammalian and avian species.

First, the effect of IFNγ on MEL production by rat pineal gland in culture *in vitro* was demonstrated [61]. This influence seems to be rather complex, because IFNγ itself enhanced NAT activity stimulated via β- adrenergic receptors of pinealocytes but isoproterenol prevents this stimulation. Thereafter, it was found that in rat IL-1β in a dose-dependent way decreased serum MEL level, and this effect was abolished by anti-IL-1 receptor antibody [62], whereas colony stimulating factors (G-CSF and GM-CSF) secreted mainly by macrophages, stimulated MEL synthesis both *in vivo* and *in vitro* [63]. On the other hand, embryonic chicken bursectomy diminished the humoral immune response as well as MEL level increased by multiple immunization, and both effects were reversed by the two injections of very low doses of the bursin into the embryo [23]. Single immunization with SRBC caused the season-, age- and gender-dependent changes in nocturnal NAT activity in chicken [64].

Therefore, there is no doubt that beside information on external lighting conditions, the pineal gland is able to perceive messages coming from immune system, most probably via soluble factors, i.e. cytokines, and therefore, close a regulatory loop between other systems, involved in the keeping of homeostasis.

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