

REVIEW

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SEASONAL CHANGES IN MELATONIN AND IMMUNOLOGICAL ADAPTATIONS IN BIRDS

HALDAR C and SINGH S S

Pineal Research Laboratory, Department of Zoology, Banaras Hindu University, Varanasi - 221005, India.

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SUMMARY

Birds in general have not been frequently examined for the immunological influences of melatonin or pineal gland, as it has been potentially noted with the mammals including human being. There are only few reports suggesting that melatonin may play some role in the development and function of the immune system in the birds. The diurnal pattern of the melatonin synthesis is the same in almost all vertebrate groups so far examined, though differences exist in structure or anatomy of the pineal gland and seasonal variation in the peripheral melatonin level. Since functional relationship between neuroendocrine pineal gland and immune systems appears as to be diverse, it is logical that immunomodulatory effects of melatonin may be different in different seasons. The seasonal variation in melatonin level has been proved to improve the status of immunity in seasonal tropical birds to bear the natural challenges. The level of peripheral melatonin minimizes some inflammatory diseases and sudden death syndrome in some poultry birds. The effect of melatonin also depends upon the phases of the inflammatory reaction/process. Pinealectomy in birds demonstrated functional interrelationship between the development of pineal gland, ontogeny of the melatonin secretion and immune system and/or neuroendocrine network. Bursectomy could influence the diurnal rhythm of melatonin and may abolish the effect of immunization on serum melatonin level. Specificities of avian immune system may be, however, very useful for understanding numerous phylogenetic and evolutionary mysteries. In this way a reciprocal functional connection between the avian immune system and the pineal gland seems to exist, but the mechanisms involved in maintaining the immune status by melatonin in avian group is still not clear and needs to be elucidated.

Key words : birds; immunity; melatonin; pineal; reproduction.

*Correspondent author, e-mail : chaldar@banaras.ernet.in

INTRODUCTION

The pineal gland is known as a regular of seasonal changes in physiological functions of several species of vertebrates (1). It is a neuroendocrine gland responsible for the transformation of external signals, mainly photoperiodic information, into a hormonal output, interpreted thereafter by internal target structures and release of its principal hormone, melatonin in a rhythmical fashion, characterised by a low level during the day and an elevation during the night.

In birds, the immune system is highly complex but well regulated through various processes, which are poorly understood. Specificity of avian immune system is however very useful for understanding numerous phylogenetic and evolutionary mysteries of them. It is now increasingly recognised that immune and neuroendocrine functions cooperate in a closely interwoven network to protect the organism from various environmental attacks (Fig. 1). Further, a number of experimental evidence exists for bi-directional interactions of immune system with the nervous systems (2-4). Products of the immune system like lymphokines and monokines modulate the neuroendocrine functions - while various neuroendocrine signals also affect the immune functions. Neuroendocrine and lymphoid cells share a number of neurotransmitters, neuromodulatory substances, hormones and their receptors in common, supporting the existence of bi-directional regulation between the two systems (5-7). Evidences also show that a reciprocal interaction exists between the pineal gland and the immune system in some vertebrate groups. It is widely held that the immunendocrine network affects the susceptibility to various diseases including cancer (8).

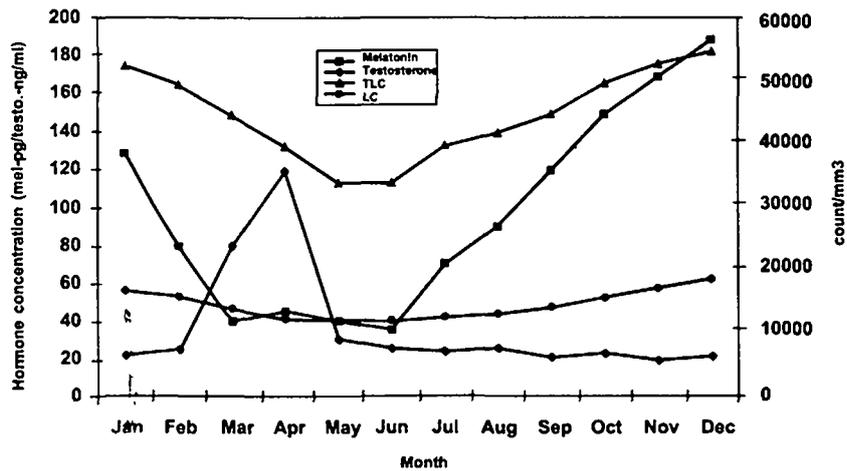


Fig.1. Annual variation in hormonal concentration of melatonin (pg/ml), and testosterone (ng/ml) along with total leukocyte count (TLC/mm³) and lymphocyte count (LC/mm³) in circulation (upper panel). Lower panel shows annual variations in biometeriological parameters (photoperiod in hours(H), temperature (0° C) and humidity (%)).

In recent years a number of endocrine and neuroendocrine circuits has been implicated in modulation of the immune system. These include not only the gonadal and adrenal hormones, but also a vast number of neuropeptides as ACTH, β - endorphin, prolactin, growth hormone and the enkaphalins. These opioids may exert either stimulatory or inhibitory effects upon immunity, implicating them as part of the regulatory circuits, and this way, the central nervous system influences the course of body's immune system. But very recently, evidence has started emerging that it is not only the CNS which influences the host defense, but also the products of lymphocytes and thymic epithelial cells have the capability to modify neuroendocrine circuits. Therefore, communication between neuroendocrine and immune system appears to include :

- 1 the uses of common signal (cognitive and non-cognitive stimuli), and
- 2 recognition molecules (IL1, IL2, γ - INF, TNF, thymic protein etc.), and the feed back system operates in bi-directional way (Fig.2)

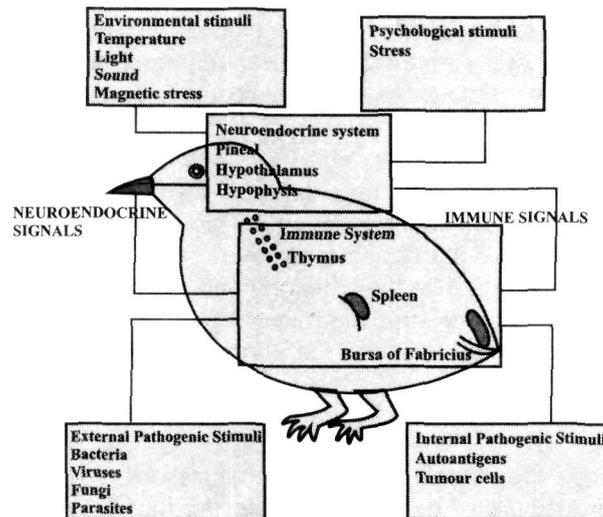


Fig. 2. A schematic view of the interaction between the cognitive, non-cognitive stimuli, neuroendocrine and immune system in birds

An immunomodulatory role for melatonin has been reported in various mammals including human being (9-17). However, the avian immunomodulation has been the subject of study in recent years and too only in domestic avian models e.g. chicks and Japanese quails (18,19).

The present review aims at presentation of consolidated information on immune system in birds and the role of melatonin on the physiological function under specific headings on the basis of the priorities in the investigation series.

Immune system in birds

In 1956, a milestone in the development of modern immunology was the discovery of the avian cloacal gland, bursa of Fabricius by Bruce Glick, from the Ohio State University,

as a lymph gland containing lymphoid follicles and epithelial cells. In terms of both growth and histological structure it resembles thymus and therefore was nicknamed as "a cloacal thymus."

Bruce Glick also demonstrated the existence of interrelationship between the weight and development of the bursa and tests. He also found that the strains of chicken with heavier body weight having more developed bursa (e.g. White Leghorn vs. Rod Island Red), were more resistant to bacterial infection e.g. *Salmonella pullorum* (20). Thereafter, it was demonstrated that the surgical bursectomy interferes with the development of antibody synthesis and exerted a suppressive effect on normal ontogeny of immune response (21). Avian bursa of Fabricius thus, emerged as a primary lymphoid gland responsible for the normal development of antibody synthesis. The lymphocytes produced by bursa were named as the B-cells in all vertebrate species, while the bursa of Fabricius is a lymphoid gland existing only in the avian species.

The bursal follicles create special environment where the B-cell precursors undergo maturation and start to express the surface immunoglobulin markers. Additionally, the bird's lymphatic organ also offered many methodological advantages, e.g. anatomical separation of the two primary lymphatic primordia or extramammary development facilitating a manipulation on the developing embryo for immune researcher. A comparative research revealed that the mammalian and avian immune systems exhibit the same basic anatomical and functional organisation i.e. fundamental dichotomy into cellular, a thymo-dependent and humoral, i.e. a bursa - or bursa-equivalent-dependent immunity. This dichotomy observed not only in higher vertebrates (endothermic i.e. birds and mammals) but also in lower ones (ectotherms i.e. fish, amphibians and reptiles). While the histology and function of the thymus is relatively similar in both mammals and birds, the structure responsible for the humoral immune response as well as immunoglobulin secreted in these vertebrates are quite different.

Importance of avian Bursa in immune function

The B-cells undergo the development and maturation in bursal microenvironment composed with the different type of cells and soluble products. One of these factors was recognised as a low-molecular bursal hormone, named "bursopoietin or bursin", a tripeptide LYS-HIS-GLY-NH₂ that induces the development of B-cells from their avian and mammalian precursors *in vitro*² (22). Recently, it was demonstrated that early embryonic bursectomy not only diminished chicken humoral immune response but also influenced the circadian rhythm of pineal gland function (pineal NAT activity, serum melatonin level) as well as the melatonin response to multiple immunization with porcine thyroglobulin (Tg) (23, 24). Both effects were reversed by bursin injection twice into the bursectomised avian embryo in very low (femtomolar and lower) doses, indicating again the existence of the functional connection between immune system and pineal gland function in birds.

Effect of Pinealectomy

The first experimental approach to demonstrate reciprocal interdependence between the pineal gland and immune was to ablate the former in order to examine the development of the latter. Reports have suggested that surgical pinealectomy may exert a stimulatory, inhibitory or no effect on immune function in the mammalian species examined to date.

Several authors demonstrated that pinealectomy affects various avian circadian rhythms, locomotor activity, thyroid gland function, and peripheral hormone levels and serotonin metabolism in the hypothalamus (25), hence effect of pinealectomy on immune system was also suspected. Effect of pinealectomy on the diurnal rhythms of the nonspecific immune parameters in the chickens was noted as a shift of granulocyte number acrophase and abolition of circadian rhythmicity of serum lysozyme concentration (26). These studies suggested that effect of pinealectomy on the development of immunity is equivocal and strongly depends on species and stage of ontogeny when the surgery was performed. A surgical pinealectomy during early embryonic development in mammals is methodologically difficult, if not impossible, while the avian embryo offers an excellent model for this kind of the study. There are several reasons for this, and the most important are that :

- 1) the avian embryo develops without direct influence from the mother organism;
- 2) its nervous, endocrine and immune system develop almost simultaneously, and
- 3) their immune system contains two separated primary lymphoid glands: thymus, comparable with that in mammals, controlling cell mediated immune response, and existing only in birds, bursa of Fabricius, a lymphoid gland furnishing cells which participate in humoral immune response.

Chicken embryo pinealectomised at 96 hrs of incubation can develop lymphoid organs properly whereas Jankovic and co-workers (27) found a retarded development and decrease cellularity of both the thymus and bursa of Fabricius. A decreased humoral immune response has been measured by plaque forming cell (PFC) number in the bursa and spleen as well as a diminishing of several parameters of cell mediated immunity following embryonic pinealectomy in chick (27). These effects were also accompanied by the significant changes in the concentrations of biogenic amines (serotonin, dopamine and noradrenaline) in the spleen, brain and hypothalamus. These results clearly proved the existence of functional relationship between the pineal gland and the development of immune system and/or neuroendocrine-immune network in avian groups as was noted in mammals. In certain cases of pinealectomy in birds, the effect may be compensated latter by melatonin coming from extra-pineal sources, and therefore, different effect may be observed in the latter stage of postnatal development.

Seasonal changes in avian pineal gland activity and immune system

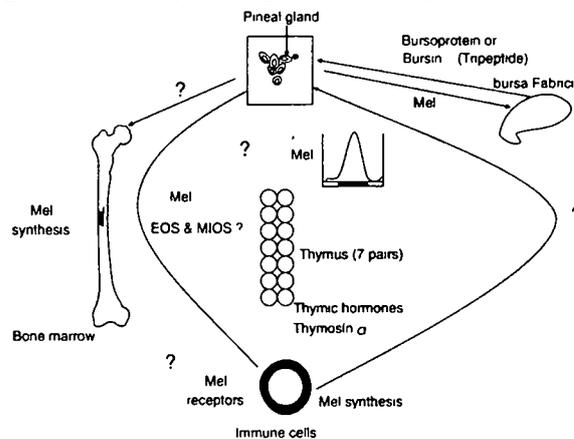


Fig 3. Hypothetical diagram showing interaction and mechanism of action of pineal gland, melatonin and immune system of birds

In tropical countries, there are little annual changes in photoperiods, but melatonin presents a typical rhythm with reproductive phases (28). Our reports suggest a distinct annual cycle in reproductive activity in Indian Jungle bush quail and this cycle appears to be influenced by photoperiods in combination with other ecological factors, such as humidity due to monsoon rainfall and temperature (28, 29; Fig-3). The role of melatonin in the integration of immune function, within the context of other energy-conserving seasonal adaptation including suppressed breeding, increased thermoregulatory capacities, curtailed growth needs more emphasis.

We believe that the distinct annual variation in photoperiods, along with other variables, leads to changes in pineal gland activity in most avian species and regulates annual variations in activity of lymphoid organs. Our recent study of annual cycles in Indian jungle bush quail, *P. asiatica*, showed a direct co-relation between the pineal gland and bursal activity (19) in support to the above statement. The immune status of Indian Jungle bush quail increases when photoperiod is minimum in winter along with low temperature (Fig.3). Recently, Kliger *et al.* (30) proposed that the amount of melatonin in the peripheral blood may regulate seasonal immune function, is discussed in the following sections.

Reproductive phase dependent effect of melatonin on immune status of birds

In seasonally breeding tropical birds, there are four distinct reproductive phases, i.e. reproductively active, regressive, inactive and recrudescence phases. We assessed the pineal immune interactions following the melatonin injection in Indian Jungle bush quail, during some crucial reproductive phases, when we suspected a decrease or increase in immune status of this bird in nature (for example, during the regressive phase these birds are susceptible to some dermal and eye infection.) The effects of melatonin treatment on the leukocyte counts, bursal, pineal (melatonin concentrations) and testicular activity (testosterone concentrations) have been noted in detail with this common high food value bird, *P. asiatica*. Our study was the first to indicate the immunomodulatory role of pineal gland and melatonin on both the bursa and spleen of a seasonally breeding bird Indian Jungle bush quail (19).

Effect of melatonin on avian immune response

Possible involvement of melatonin in the intrathymic education of developing T cells, as well as some role of the thymic microenvironment in melatonin message transmission between blood and immune cells have been studied in birds. Developmental changes in melatonin binding sites indicate a developmental interdependency between the immune system and the pineal gland. Density of melatonin binding sites varied with gland/organ and age: the highest variable was in the spleen, suggesting that this secondary lymphoid gland containing a more heterogeneous cellular composition in relation to sensitivity to melatonin. In immunocompetent birds, melatonin binding by both primary (thymus and bursa of Fabricius) and a secondary (spleen) lymphoid organ is unrelated to immune system activation by a T dependent antigen. Moreover, elimination of melatonin synthesis by pinealectomy profoundly decreased the proliferation of the bone marrow progenitors for granulocytes and macrophages (CFU-MG), and the night-time peak of melatonin completely abolished CFU-MG proliferation (9,10).

In chicken with intact gland, the diurnal rhythm of granulocyte number was strictly dependent on the time of melatonin injection. The diurnal rhythms of examined non-specific

immune parameters in chicken depend on pineal gland and melatonin but the involved mechanisms are different (26).

It needs to be pointed out that the studies on intact, melatonin and/or corticosterone-treated chicken were made according to the experimental protocol in which the hormone was efficient as an immunostimulatory and anti-stress agent in mice (31-33). In tropical rodent, Singh (34), reported that dexamethasone treatment decreased the cell mediated as well as humoral immune responses. Our (unpublished) data showed that dexamethasone treatment in quail lowered the circulating lymphocyte number in blood and melatonin treatment along with dexamethasone, reduced the effect of dexamethasone and restored the circulating lymphocyte number to normal. Some of the melatonin treated chicken were injected with opioid antagonists naltrexone or naloxone to examine whether the endogenous opioids system (EOS) may be involved in the melatonin effect on immunity in chicken (18), as it was found in mice (33). Both melatonin and opioid antagonists diminished spleen PFC number in chicken, whereas in the mice the stimulatory effect of melatonin was antagonized with naltrexone (35) suggesting a basic difference between opioid mechanism.

The effect of melatonin on chicken immune cells *in vitro* was examined by Markowska *et al.* (36). Melatonin alone added to the lymphocyte culture in wide range of concentration did not influence the cell proliferation, measured by the ³H-thymidine incorporation. In culture stimulated with the common T-cell mitogens, melatonin addition generally diminished the cell proliferation and the effect was best seen in the cultures of lymphocytes isolated from the youngest chickens examined (5-days-old). Melatonin added to the cell culture simultaneously with the mitogen or 2 hours earlier, exerted similar effect on lymphocyte proliferation. But, when the splenocytes were pretreated with the mitogen for two hrs. melatonin addition blocked almost completely the blastogenic reaction (36). It has also been suggested that Ca²⁺ is involved in the early lymphocyte activation of chick embryo (37).

Effect of immunization on the avian pineal gland function

Youbicier Simo *et al.* (23) demonstrated an increase in the diurnal serum melatonin concentration after second antigenic challenge. These effects exerted by single immunization with sheep red blood cells (SRBC) were noted to be dependent on the sex and season. In both sexes of chicks a nocturnal N-acetyltransferase activity was negatively correlated with serum anti-SRBC antibody level in winter, whereas a similar effect in spring was seen only in females. In males, the same immunization evoked a less pronounced effect in spring, but there was rather a positive correlation between both parameters. There are several possible explanations for this difference in reaction of the chicken pineal gland to immunization. The most important one seems to be the kind of antigen used (a particulate SRBC vs. soluble Tg), number of antigenic stimulation (single vs. multiple), and parameters measured (nocturnal pineal NAT activity vs. diurnal serum melatonin level). Moreover, we have no information about the sex of the chicken examined and the season in which the experiments with Tg immunized birds were performed (23). Such studies are totally lacking for any seasonally breeding birds, which show sensitivity to seasonal infections and immune status.

Melatonin receptors within the avian immune system

A number of studies have now confirmed the existence of melatonin receptors in lymphatic tissues, as well as on circulating cells of the immune system. Binding sites on

splenocytes were located in the cell nucleus, rather than membrane, and display reversibility, high affinity, specificity and light sensitivity, as well as time and temperature dependency. Yu and co-workers (38) were the first to demonstrate their presence in avian spleens with higher density in chicken and ducks than in mice. Subsequently, specific reversible and high specific binding of 2-[¹²⁵I] iodomelatonin was described in membrane preparations from avian spleen (chicken, pigeon, quail; (39, 40), thymus (duck) and bursa of Fabricius (duck; 39,41-44). Melatonin binding sites in avian lymphoid glands fulfill all functional criteria suggesting that they belong to the M1 class of melatonin receptors proposed by Dubocovich (45). In 4 week-old cockerels melatonin binding by membrane preparations isolated from whole lymphoid glands was much lower than in the brain (46). Comparatively, among all the lymphoid tissues the highest 2-[¹²⁵I] iodo-melatonin binding was found in the bursa of Fabricius, much lower in spleen and only traces in the thymus.

Melatonin binding sites have also been found on human lymphoid cells and T-helper 2 lymphocyte in the bone marrow of vertebrates. The melatonin receptors on lymphatic tissue seem to be coupled to G-protein. Activation of these putative melatonin receptors with both physiological and pharmacological concentration of melatonin evoked production of interleukin-4, which induces hematopoietic growth factors secretion from bone marrow stroma cells. Further melatonin has also been shown to enhance interleukin-2, interleukin-6 and γ -interferon production by human circulating CD4+ cells. However in birds neither Kd nor density of melatonin binding sites in chickens could be modified by immunization with SRBC, which caused a significant immune response, as measured by the serum anti-SRBC agglutinin level. Therefore, it is suggested that in immunocompetent birds, melatonin binding by both primary (thymus and bursa of Fabricius) and secondary (spleen) lymphoid organs is unrelated to the immune system activation by a T-dependent antigen (18). However, these results are in disagreement with the results obtained by Poon *et al.* (44) in 2-week old chicks treated for seven days with pharmacological doses of cortisol, where a significant reduction of Bmax was observed. This change was in the immune status of cortisol-treated birds in comparison to control. Further, Wang *et al.* (40) have reported a significant increase in the number of melatonin binding sites on spleen membrane preparations hydrocortisone-treated pigeons. We think it is worthwhile to stress upon that when membranes were prepared from isolated lymphocytes there remained some tissue debris containing epithelial cells, blood vessels and, probably, some remained lymphocytes, both subfractions of respective lymphoid glands which also exhibited ability to bind melatonin. These results imply the possible participation of melatonin in the intratissue microenvironment formation and/or development and maturation of immunocompetent cells.

CONCLUSION

Melatonin plays an important immunoregulatory role by both direct and indirect action on the avian immune system. Reports available to date suggest that in chickens, as in mice, melatonin may be involved in the development, maturation and function of the immune system, but mechanism(s) operating may be different. However, in birds the immune system and melatonin may also operate via endogenous opioids, but the effect exerted by endogenous opioid system appears to be different than mammals. In summary, melatonin is a part of an integrative system to coordinate reproductive, immunologic and other physiological processes to cope successfully with energetic stressors during winter of birds (Fig.3).

It remains to be established whether or not melatonin binding sites within immune system may participate directly in the immunomodulatory activity of this hormone, but gives the first indication that melatonin by a modulatory influence on its own binding site, may act directly upon the function of immune system.

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