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Melatonin blocks dexamethasone-induced immunosuppression in a seasonally breeding rodent Indian palm squirrel, *Funambulus pennanti*

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Abstract

In vivo effect of dexamethasone and melatonin on immunomodulation has been investigated by studying the lymphocyte proliferation to the mitogen Con A from various lymphoid tissues including bone marrow cells of a seasonally breeding rodent adult male *F. pennanti* during reproductively inactive phase (October to December). During this phase, animal faces the maximum challenges of the nature (hypothermic stress, scarcity of food and shelter). Dexamethasone treatment (60 μg/day/squirrel) for 60 consecutive days significantly decreased the thymus and spleen activity. The lymphoid tissues mass, total leukocyte, lymphocyte count of peripheral blood, bone marrow and T-cell mediated immune function was also significantly suppressed following the dexamethasone treatment but treatment of melatonin (25 μg/squirrel/day) along with dexamethasone significantly restored the suppressed immune status in squirrels. Further, histological study of the thymus showed profound changes in the cellularity with a depletion of thymocytes in the cortex region of thymic lobules and increased in connective tissues and spindle cells. Melatonin treatment alone increased thymocytes density in thymic cortex, clearly suggesting that melatonin counteracted the experimentally induced immune stress by dexamethasone. Therefore, in nature during reproductively inactive phase of the squirrel a high level of melatonin was noted, that is required to combat nature's stress, which might have increased the internal level of corticoids.

Keywords: Melatonin; Dexamethasone; Immunity; *Funambulus pennanti*; Seasonal stress

1. Introduction

Interactions between glucocorticoids and immune function have been reported in relation to environmental stress. Adrenocortical hormones, especially glucocorticoids, suppress immune function in both humans and non-humans [1] and were found necessary for growth of cultured lymphocyte [2]. Reports exist to demonstrate that environmental stresses elevate blood glucocorticoid levels and that high glucocorticoid levels suppress cellular and humoral immune function [3,4] and increases susceptibility to both infections and neoplastic processes [5]. Corticosterone has been reported to suppress antibody production, nucleated spleen cells and causes thymic atrophy [6].

Further, melatonin, a principal pineal neurohormone has been reported to ameliorate the immunocompromising effects of glucocorticoids [7,8]. In contrast, melatonin has been reported to cause immune suppression in human [9]. However, nothing is known about how circulating melatonin can help the seasonal breeders to protect them from environmental stress. It is important to note that during reproductively inactive phase (October to December) of male Indian palm squirrels, environmental temperature is low (hypothermic) with scarcity of food grains and shelter in nature. We have published elsewhere that in Indian Jungle bush quail, *Perdicula asiatica* under such condition with high melatonin level enhance immune status [10]. However, it has never been accessed for seasonally breeding tropical rodents as temperature and humidity differences are maximum than photoperiod in tropics. Therefore, the present investigation was undertaken to elucidate the immunomodulatory role of pineal hormone melatonin in relation to dexamethasone, which is a potent synthetic corticosterone in a seasonally breeding rodent, adult male Indian palm squirrel, *Funambulus pennanti* during reproductively inactive phase. Reproductively inactive phase is very crucial for this rodent since it has to fight with environmental stress

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(low temperature/less food availability/lack of shelter) with low steroid level. Another reason for doing this experiment in reproductively inactive phase was to establish the adaptive significance of melatonin in preventing animal from winter stress.

2. Experimental

Adult male squirrels approximately of same age as judged by their cranium diameter and incisor length [11] and weight (120 ± 5 g) were collected from the vicinity of Varanasi (latitude 25°18′N and longitude 83°1′E) during the second week of October (November being the reproductively inactive phase). They were weighed and kept in wire net cages (25 in. × 25 in. × 30 in. in size) exposed to natural day length (11 h light:13 h dark) and temperature (minimum: ∼15 °C, maximum: ∼28 °C) for acclimatization. They were fed with soaked gram seed (*Cicer arietinum*) and water ad libitum. The final body weight of the squirrels at the end of the experiment in November had no significant difference from the initials. All the experiments on the animals were conducted in accordance with Institutional practice and within the framework of revised Animals (Specific Procedure) Act of 2002 of Government of India on animal welfare.

2.1. Drugs and treatment protocol

Dexamethasone (Dex), melatonin (Mel) and mitogen Concanavalin A (Con A) were purchased from Sigma. Chem. Co. (St. Louis, USA). Melatonin and dexamethasone solution was made by dissolving it in few drops of ethanol (10%) and then diluted in normal saline (0.9% NaCl) up to desired concentration. After 15 days of acclimatization (end of October) to the laboratory condition the squirrels were randomized in the following four groups each containing 12 squirrels.

Group	
I	Veh Con
II	Dex-treated
III	Dex + Mel
IV	Mel-treated

Dexamethasone (60 μg/squirrel/day) [12] and melatonin (25 μg/squirrel/day) treatment were given subcutaneously during the evening hours (4.30 to 5.00 p.m.) for 60 consecutive days till the end of the reproductive inactive phase, i.e. November and December (11 h light:13 h dark and temperature, minimum: ∼11 °C, maximum: ∼20 °C in November and in December minimum: ∼5 °C, maximum: ∼10 °C). Twenty-four hours after the last injection six squirrels from each group was subjected for Delayed Type Hypersensitivity (DTH) response assay to oxazolone (T-cell antigen) following the ear swelling tests of Phanuphak et al. [13] with slight modification. Remaining six squirrels were subjected for lymphoid organs weight analysis and blastogenic response of lymphoid cells following their sacrifice at evening hours (5.00–6.00 p.m.). Total leukocyte count (TLC) was assessed in hemocytometer using Turk's solution. Differential count (DLC) for eosinophils, neutrophils and basophils was determined from TLC. For bone marrow lymphocytes femur bones of both the legs were dissected out and bone marrow strip was flushed with the help of syring along with phosphate buffer solution in a sterilized test tube. Bone marrow strip was then well agitated to make a homogeneous cell suspension. A small drop of this suspension was put on the clean slide and spreaded to make a thin film, which was allowed to dry. The slide was stained with Leishman stain, observed in microscope and percentage of lymphocyte were calculated.

RIA of melatonin was done following the method of Attanasio et al. [14]. Percent recovery after extraction was 92%. The intra-assay and inter-assay CVs were 9 and 15%, respectively. The sensitivity of the assay was 10 pg/ml for 200 samples. Plasma coticosterone was estimated by spectroflurometric assay following the method of Mattingly [15]. Percent recovery after extraction was 85%. The intra-assay and inter-assay CVs were 5.5 and 8.5%, respectively. The sensitivity of the assay was 25 ng/ml.

2.2. Histology

Thymus was dissected out and fixed in Bouin's fluid. Histological section of 5 μm thick were cut and then stained with hematoxylin and eosin. Representative photographs of each group of thymus were taken at Leitz MPV3 microscope under (40×) magnification.

2.3. Blastogenic response to mitogen

The blastogenic response to 4.5 μg/ml of the mitogen Con A was evaluated following the method of Pauly and Sokal [16]. The mononuclear lymphoid cells (1×10^6 cells/ml) were incubated in medium in a plastic 96-well tissue culture plate for 72 h. The lymphoid cell proliferation was assayed by pulse labeling with tritiated thymidine (^{3}H-TdR; specific activity 8.9 Ci mM; BARC Mumbai, India), 18 h before the end of incubation period. A 0.1 ml aliquot was counted using a liquid scintillation counter (Packard, USA). Results are expressed as ^{3}H-TdR incorporation in counts per minute.

2.4. Statistical analysis

Statistical analysis of the data was performed with one-way ANOVA followed by Student–Newman–Keuls test. The differences were considered significant when $P < 0.05$.

Table 1
Variations in different blood parameters (total leukocyte, percent lymphocyte count of blood and bone marrow) percent count of eosinophils, basophils, neutrophils of *F. pennanti* following dexamethasone and melatonin injection alone and in combination

Parameters	Experimental groups			
	Veh Con	Dex-treated	Dex + Mel	Mel-treated
Total leukocyte count (TLC)	8925 ± 1387.44	7700** ± 739.50	9895** ± 1268.41	12080** ± 840.83
Lymphocyte count	2268 ± 98.25	1521** ± 279.44	2346.6** ± 362.08	3589.8** ± 212.24
Percent lymphocyte count (bone marrow)	9 ± 1.4	5.2** ± 1.16	7.8** ± 0.7	11.6** ± 1.09
Eosinophils (%)	3 ± 0.11	3.5 ± 0.2	2.5 ± 0.13	3.1 ± 0.12
Basophils (%)	1 ± 0.09	1 ± 0.085	1.2 ± 0.1	1.5 ± 0.09
Neutrophils (%)	58 ± 3.5	38** ± 4.2	65** ± 3.9	48** ± 4

Data presented mean ± S.E.M.
** $P < 0.01$ Veh Con vs. Dex, Dex vs. Dex + Mel, Veh Con vs. Mel.

3. Results

3.1. Lymphoid organs weight

3.1.1. Thymus, spleen and mesenteric lymph nodes (LN)

Dexamethasone treatment significantly ($P < 0.01$) decreased the thymus, spleen and LN weight when compared with control squirrels. Melatonin treatment along with dexamethesone antagonized the dexamethasone-induced suppression of lymphoid organs weight and showed significant ($P < 0.01$) increase of thymus, spleen and LN weight when compared with Dex-treated group. Moreover, treatment of melatonin to the squirrels showed a significant ($P < 0.01$) increase of thymus, spleen and LN weight when compared with control group (Figs. 1A, B and 2).

3.2. Effect on hematological parameter (TLC, LC, and DLC)

3.2.1. Total leukocyte and lymphocyte count (TLC and LC)

Administration of dexamethasone caused a significant ($P < 0.01$) decrease of total leukocyte and lymphocyte counts when compared with control group. Further, melatonin treatment along with dexamethasone showed restored it upto the control level when compared with Dex-treated group. Melatonin treatment alone to the squirrels enhanced the total leukocyte and lymphocyte counts significantly ($P < 0.01$) when compared with control group and similar observation was also noted for the percentage lymphocyte count in bone marrow and percent lymphocyte count of peripheral blood as well (Table 1).

3.2.2. Percent count of neutrophils, eosinophils and basophils

Dexamethasone-treated group of squirrels showed a significant increase ($P < 0.01$) in percent of neutrophils but a combined treatment of dexamethasone and melatonin however, decreased neutrophils percentage. Further, significant decreases in the percent count of neutrophils were noted in the melatonin-treated squirrels as compared with that of the control group of squirrels. Dexamethasone treatment alone and in combination with melatinin could not affect the percent count of eosinophils and basophils (Table 1).

3.3. Blastogenic responses of thymocytes, splenocytes and lymph node (LN) cells

Dexamethasone treatment decreased the mitogen Con A induced blastogenic response (counts per minute) of thymocytes splenocytes and LN cells significantly ($P < 0.01$) when compared with control group. Further, melatonin treatment along with dexamethasone significantly ($P < 0.01$) enhanced the dexamethasone suppressed basal blastogenesis of thymocyte, splenocytes and LN cells. In addition, melatonin treatment alone significantly ($P < 0.05$) enhanced the basal blastogenesis of thymocytes but did not show significant change of splenocytes basal blastogenesis. A significant ($P < 0.01$) increase of basal blastogenesis in lymph node cells were noted when compared with control group (Figs. 3A, 4A and 5A).

3.4. Percent stimulation ratio (%SR) of thymocytes, splenocytes and lymph node cells

Dexamethasone-treated animals showed a significant ($P < 0.01$) decrease of %SR when compared with control group. Further, combined treatment of melatonin and dexamethasone significantly ($P < 0.01$) enhanced the dexamethasone suppressed %SR of thymocytes, splenocytes and LN cells. Further, melatonin treatment had no effect on of thymocytes and splenocytes %SR, while lymph node cells showed significant ($P < 0.01$) increase of %SR when compared with control group (Figs. 3B, 4B and 5B).

3.5. Delayed Type Hypersensitivity (DTH) response to oxazolone

The DTH response to oxazolone was measured in terms of ear thickness following the method of Phanuphak et al. [13]. The dexamethasone treatment to the squirrels significantly ($P < 0.01$) suppressed the DTH response to oxazolone when compared with control. Melatonin treatment

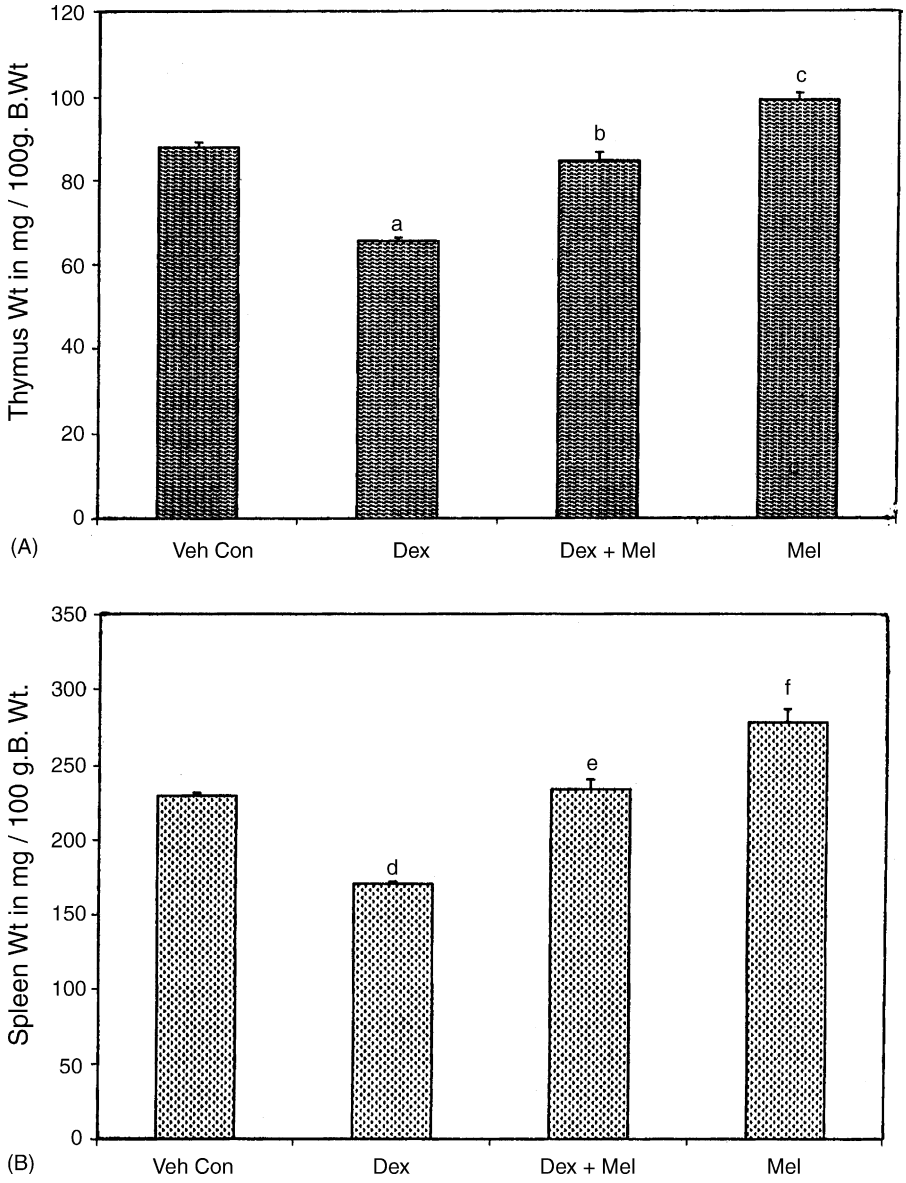

Fig. 1. Effect of dexamethasone and melatonin treatment on (A) thymus and (B) spleen weight in seasonally breeding rodent, Indian palm squirrels during reproductive inactive phase (November to December). Histograms represent mean ± S.E., $n = 6$ for each group within this experiment. Veh Con: vehicle-treated control, Dex: dexamethasone, Mel: melatonin. (a and d) $P < 0.01$ Veh Con vs. Dex, (b and e) $P < 0.01$ Dex vs. Dex + Mel, (c) $P < 0.01$ Veh Con vs. Mel, (f) $P < 0.05$ Veh Con vs. Mel (Student–Newman–Keuls test).

along with dexamethasone showed a significant ($P < 0.01$) increase of the DTH response. Melatonin treatment alone significantly ($P < 0.01$) increased the DTH response when compared with control group (Fig. 6).

3.6. Plasma melatonin and corticosterone level

Subcutaneous dexamethasone treatment during evening hours (4.30–5.00 p.m.) for 60 consecutive days showed a significant decrease ($P < 0.01$) of plasma melatonin level when compared with control group while a significant increase ($P < 0.01$) was noted in plasma corticosterone level. Further, cotreatment of dexamethasone and melatonin showed an increase of plasma melatonin concentration whereas decrease in plasma corticosteroid significantly ($P < 0.01$) when compared with Dex-treated group. However, exogenous melatonin treatment showed an increase of the plasma melatonin and inversely decrease ($P < 0.01$) in plasma corticosterone concentration when compared with control group (Fig. 7A and B).

3.7. Thymic cellularity following dexamethasone and melatonin treatment

Dexamethasone treatment caused profound changes in histology of the thymic cortex as well as in medulla region. Thymocytes density was severely depleted and connective tissue and spindle cells increased in thymic lobules

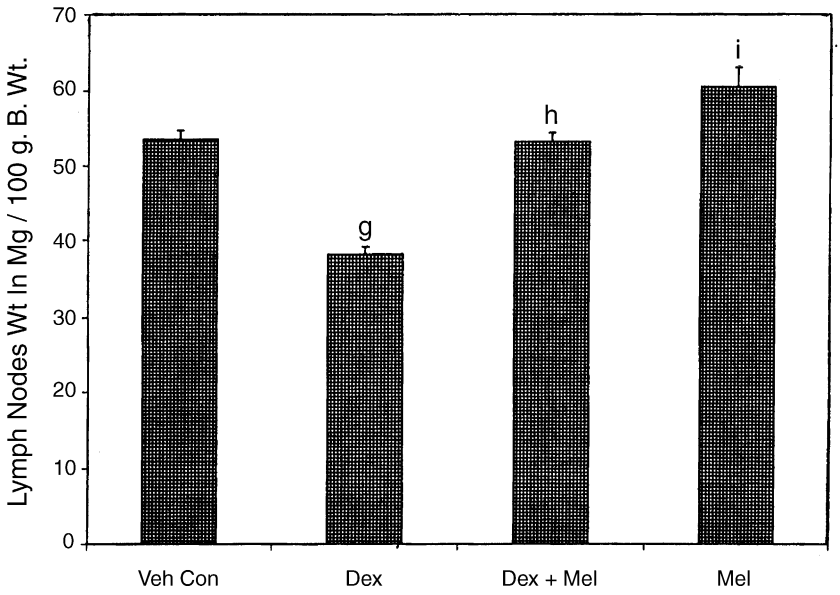

Fig. 2. Effect of dexamethasone and melatonin treatment on mesenteric lymph nodes weight in seasonally breeding rodent, Indian palm squirrels during reproductive inactive phase (November to December). Histograms represents mean ± S.E., $n = 6$ for each group within this experiment. Veh Con: vehicle-treated control, Dex: dexamethasone, Mel: melatonin. (g) $P < 0.01$ Veh Con vs. Dex, (h) $P < 0.01$ Dex vs. Dex + Mel, (i) $P < 0.05$ Veh Con vs. Mel (Student–Newman–Keuls test).

when compared with control squirrel's thymic cellular architecture. Melatonin treatment along with dexamethasone antagonized the dexamethasone-induced changes in thymic cell number. Moreover, melatonin treatment increased the thymocyte density in thymic cortex as well as in medullary region when compared with control thymic cell numbers (Fig. 8).

4. Discussion

Our animal model *F. pennanti* is a seasonal breeder of tropical Indian origin. The breeding cycle and its interrelationship with pineal gland and melatonin has already been established [17]. This rodent faces the maximum challenges of the nature during reproductively inactive phase (November to January) when tropical ambient temperature in North India is very low (5 ± 2 °C) with high scarcity of food grains and shelter hence, the experiment was planned during this phase in order to note the immunomodulatory role of melatonin and its adaptive significance.

A functional link between the neuroendocrine and immune systems has already been proposed [18]. The data presented here regarding the lymphoid organs weight, i.e. thymus spleen and lymph nodes showed a significant decrease following the dexamethasone treatment for the 60 consecutive days. The decreases noted in lymphoid organs weight are in consonant with the earlier reports on mice, that corticosteroid treatment or stress-induced increase of glucocorticoid caused thymus and spleen involution [8,19,20]. Effect of melatonin on adrenal weight and histology of this species, suggested that melatonin injection decreased adrenal gland weight (54–34 g) and width of adrenal madullary (6.4–4.6 μm) and cortex region (cortex width 6.7–5.4 μm) [21]. Seasonal diseases like conjuctivitis and dermal infections were noted in this rodent during winter months of tropics (November to January). Further, our preliminary study with pineal–adrenal interaction suggest a high adrenal weight in winter months due to stressful condition emerging from low temperature (∼10 °C), short day length (∼10.30 °C) and lack of food grains in nature.

During reproductively inactive phase, the squirrels presented a slight increase of lymphoid mass as well as blastogenic response in comparison to the reproductively active phase. This increased lymphoid tissue activity could be due to the elevated internal melatonin level in response to environmental stress [13]. Melatonin treatment with dexamethasone antagonized the dexamethasone-induced lymphoid tissue involution. Our finding resembled with an earlier report, where melatonin counteracted the corticosteroid-induced thymic involution in mice [8]. Further, to confirm our lymphoid organ weight analysis, we performed histological observation of thymus. The dexamethasone-treated thymic cellular architecture showed a depletion of thymocytes in the thymic cortex region and other non-lymphoid cells, i.e. spindle cells and connective tissue occupied the position in thymic cortex as well as in medulla region. Thymic histological study again supported the decrease of lymphoid organ weight, i.e. thymus (Fig. 8). However, melatonin treatment at evening hours (4.30–5.00 p.m.) along with the dexamethasone prevented the dexamethasone-induced changes in thymic cellular architecture supporting our earlier observation on annual immune regulation of this squirrels [13].

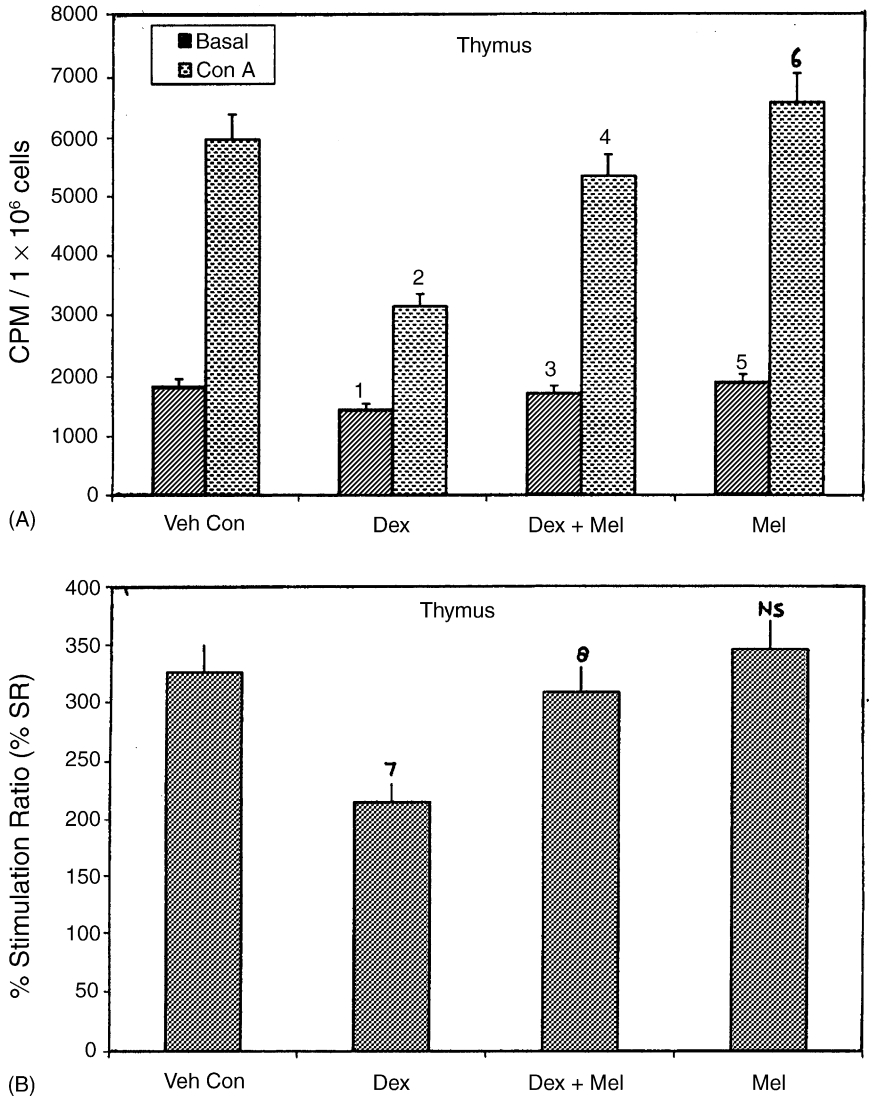

Fig. 3. Effect of dexamethasone and melatonin treatment on (A) basal, mitogen Con A induced blastogenic response and (B) percent stimulation ratio (%SR) of thymocytes in seasonally breeding rodent, Indian palm squirrels during reproductive inactive phase (November to December). Histograms represent mean ± S.E., $n = 6$ for each group within this experiment. Veh Con: vehicle-treated control, Dex: dexamethasone, Mel: melatonin; (1, 2, and 7) $P < 0.01$ Veh Con vs. Dex, (3, 4, and 8) $P < 0.01$ Dex vs. Dex + Mel, (6) $P < 0.01$ Veh Con vs. Mel, (5) $P < 0.05$ Veh Con vs. Mel, NS: Veh Con vs. Mel (Student–Newman–Keuls test).

The exact mechanism by which melatonin treatment maintained the thymic cellularity is still unknown. It has been reported that the possible reason for these changes could be that melatonin diminished DNA fragmentation induced by glucocorticoids as noted in rat thymocyte, therefore, melatonin might be able to prevent the thymocytes both from the biochemical hallmark of apoptosis and its morphological features [22–24]. This might be an explanation for the maintenance of function and cellularity in thymus after melatonin administration, and or pineal hyper-function of this rodent as noted by others during this phase of reproduction in *F. pennanti* [25,26]. Melatonin also might have protective role in stressful conditions due to its specific activation of the α_2-adrenergic receptors subtype [27].

We also studied the circulating total leukocyte, lymphocyte numbers as well as the percentage of neutrophils, eosinophils and basophils of peripheral blood and percent lymphocyte in bone marrow since, these are the important component of the immune system having high clinical value and affected the immune status of the animals. Our data showed significant suppression of circulating total leukocyte and lymphocyte numbers following the long term (60 days) treatment of the potent synthetic corticosteroid, i.e. dexamethasone. Suppression in leukocyte population was mainly due to the decreased population of lymphocytes in the blood circulation. Surprisingly, an opposite trend was noted in neutrophil count which increased significantly ($P < 0.02$) after the dexamethasone treatment whereas a significant decrease ($P < 0.02$) was noted in those group of squirrels

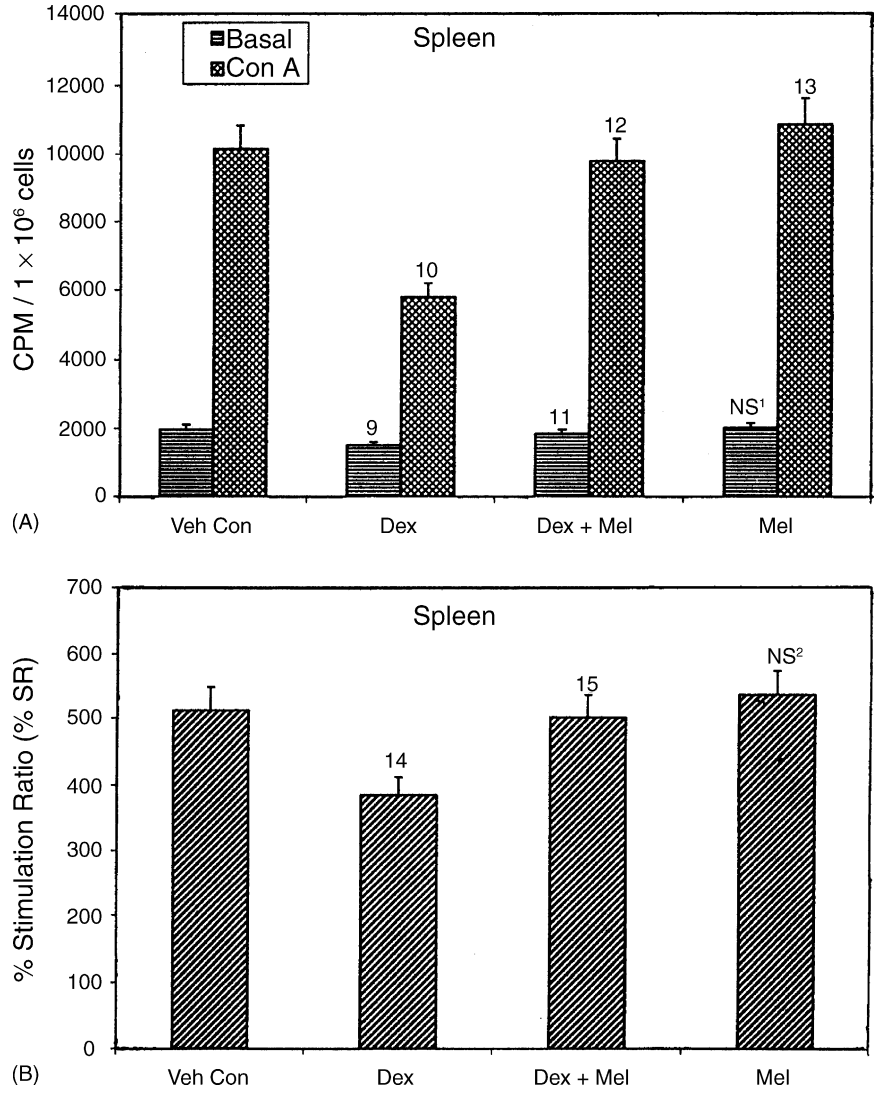

Fig. 4. Effect of dexamethasone and melatonin treatment on (A) basal, mitogen Con A induced blastogenic response and (B) percent stimulation ratio (%SR) of splenocytes in seasonally breeding rodent, Indian palm squirrels during reproductive inactive phase (November to December). Histograms represent mean ± S.E., $n = 6$ for each group within this experiment, Veh Con: vehicle-treated control, Dex: dexamethasone, M: melatonin; (9, 10, and 14) $P < 0.01$ Veh Con vs. Dex, (11, 12, and 15) $P < 0.01$ Dex vs. Dex + Mel, NS^1 and NS^2: Veh Con vs. Mel, (13) $P < 0.01$ Veh Con vs. Mel (Student–Newman–Keuls test).

treated with melatonin only due to the reason as neutrophils serves as a mobile defensive force of cells that can migrate through capillary wall in blood circulation under the any stressful condition. However, a non-significant change in counts of eosinophil and basophils was found suggesting that these blood cells tend to leave the blood stream under the influence of the synthetic glucocorticoid [28].

Interestingly, the similar pattern of observation was noted for the percentage of lymphocyte of the bone marrow when compared with the lymphocyte count for the peripheral blood. In the sham control squirrel, percent lymphocyte count was to the normal level (6–9%) whereas dexamethasone-treated squirrel presented drastic decrease in the percent lymphocyte count. When the squirrels were injected with the exogenous melatonin the percent lymphocyte increased being more than the control squirrels. However, when a combined injection of dexamethasone and melatonin was given the percent lymphocyte increased than those animals, which had dexamethasone only. This data clearly suggest that melatonin is having a lymphoproliferative action on bone marrow and most evidently melatonin injection overcome the immunosuppressive effect of dexamethasone. Previous reports on CFU-GM and role of melatonin studied in rat also supported the above study [29].

It has been reported that the exogenous corticosteroid treatment possibly enhanced the redistribution or trapping of WBC in the bone marrow [30] and this could be a reason for the decrease of leukocyte and lymphocyte noted in our study. Treatment of melatonin simultaneously with the dexamethasone counteracted the dexamethasone-induced suppression of leukocytes and lymphocyte population. Further, the peak time of immune system variables (e.g. total circulating

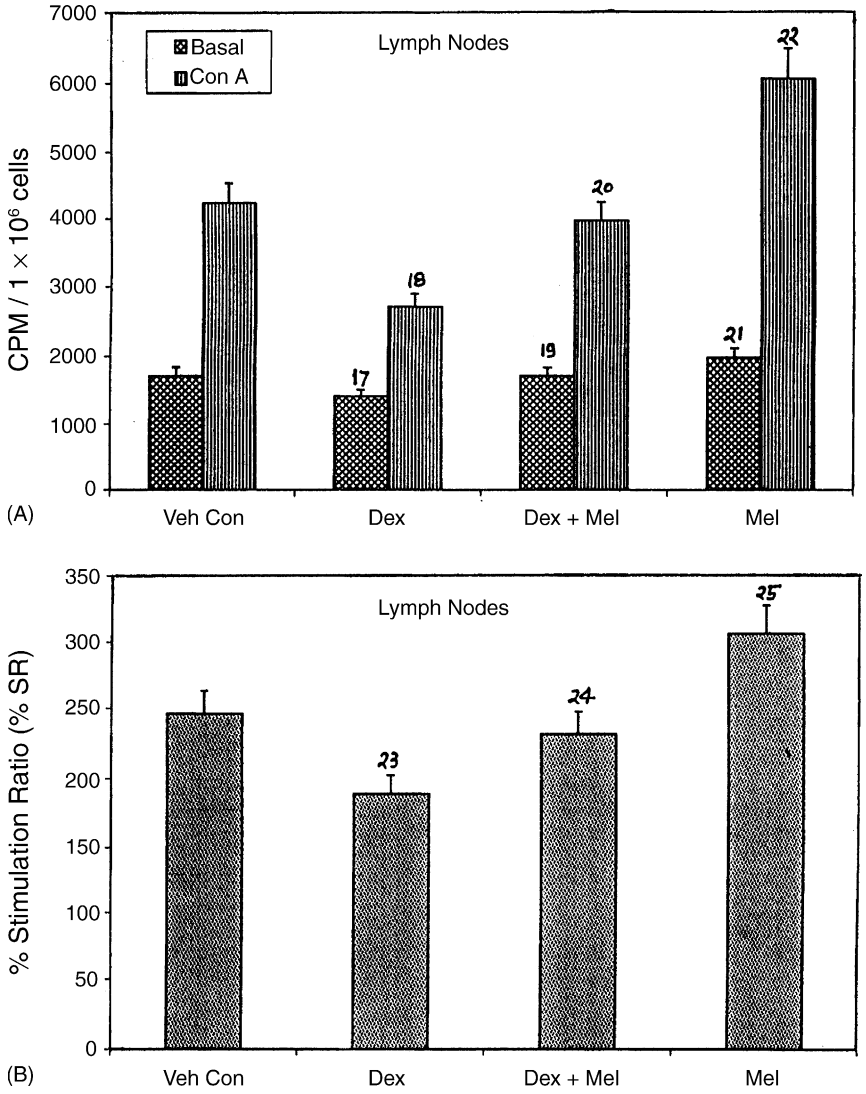

Fig. 5. Effect of dexamethasone and melatonin treatment on (A) basal, mitogen Con A induced blastogenic response and (B) percent stimulation ratio (%SR) of lymph node cells in seasonally breeding rodent, Indian palm squirrels during reproductive inactive phase (November to December). Histograms represent mean ± S.E., $n = 6$ for each group within this experiment. Veh Con: vehicle-treated control, Dex: dexamethasone, Mel: melatonin; (17, 18, and 23) $P < 0.01$ Veh Con vs. Dex, (19, 20, and 24) $P < 0.01$ Dex vs. Dex + Mel, (21, 22, and 25) $P < 0.01$ Veh Con vs. Mel (Student–Newman–Keuls test).

lymphocytes, natural killer (NK) cell activity) corresponds closely to that of melatonin [31]. Therefore, we may suggest that circulatory melatonin level might play an important role in the controlling circulating leukocyte and lymphocyte population of seasonal breeders.

Another studied immune parameter, i.e. DTH to the oxazolone, reflects the T-cell mediated immune response showed significant decrease in the DTH response following the dexamethasone treatment. However, melatonin treatment along with dexamethasone showed antagonizing effects of the dexamethasone-induced DTH response suppression.

Melatonin may counteract the immunosuppressive effects of glucocorticoids that are induced during seasons as viral infections, by inducing T-helper cells to release opiod peptides [32,33]. The immunostimulating properties of melatonin seem to depend on activation of CD4+, T-cells, which upon melatonin stimulation shows an enhanced synthesis and or release of opioid peptides, IL-2 and γ-interferon [33–35].

Our study on lymphocyte proliferation in response to the mitogen Con A showed significant suppression of thymocyte, splenocytes and lymph node cells after the long term (60 days) administration of dexamethasone. However, melatonin administration showed significant restoration in dexamethasone suppressed proliferative response of lymphoid cells.

Further, melatonin-binding sites have been described on the circulating lymphocytes [36] as well as thymocytes and splenocytes [37,38], suggesting a direct effect of melatonin on the regulation of the immune system [39,40]. However, modulatory effects of dexamethasone and melatonin on immune function may be explained in terms of receptor density

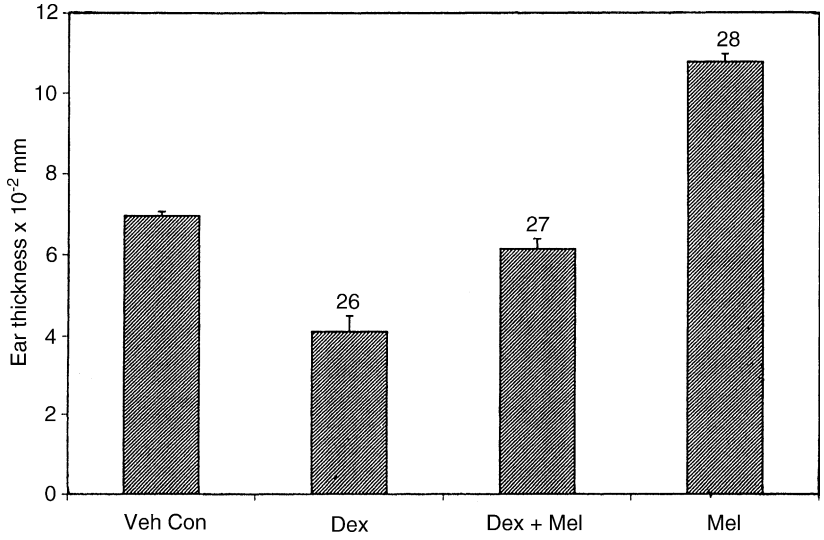

Fig. 6. Effect of dexamethasone and melatonin treatment on the DTH response to oxazolone (in terms of ear thickness) in seasonally breeding rodent, Indian palm squirrels during reproductive inactive phase (November to December). Histograms represent mean ± S.E., $n = 6$ for each group within this experiment. Veh Con: vehicle-treated control, Dex: dexamethasone, Mel: melatonin; (26) $P < 0.01$ Veh Con vs. Dex, (27) $P < 0.01$ Dex vs. Dex + Mel, (28) $P < 0.01$ Veh Con vs. Mel (Student–Newman–Keuls test).

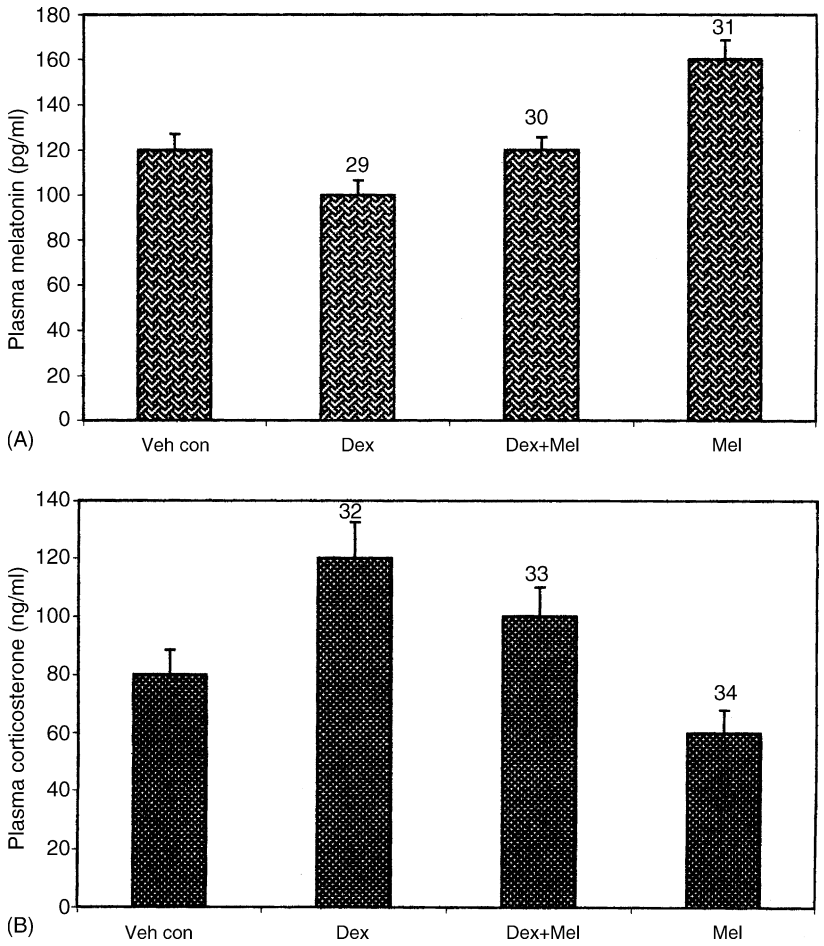

Fig. 7. Effect of dexamethasone and melatonin treatment on (A) plasma melatonin (pg/ml) and (B) plasma corticosterone (ng/ml) in seasonally breeding rodent, Indian palm squirrels during reproductive inactive phase (November to December). Histograms represent mean ± S.E., $n = 6$ for each group within this experiment. Veh Con: vehicle-treated control, Dex: dexamethasone, Mel: melatonin; (29 and 32) $P < 0.01$ Veh Con vs. Dex, (30 and 33) $P < 0.01$ Dex vs. Dex + Mel, (31 and 34) $P < 0.01$ Veh Con vs. Mel (Student–Newman–Keuls test).

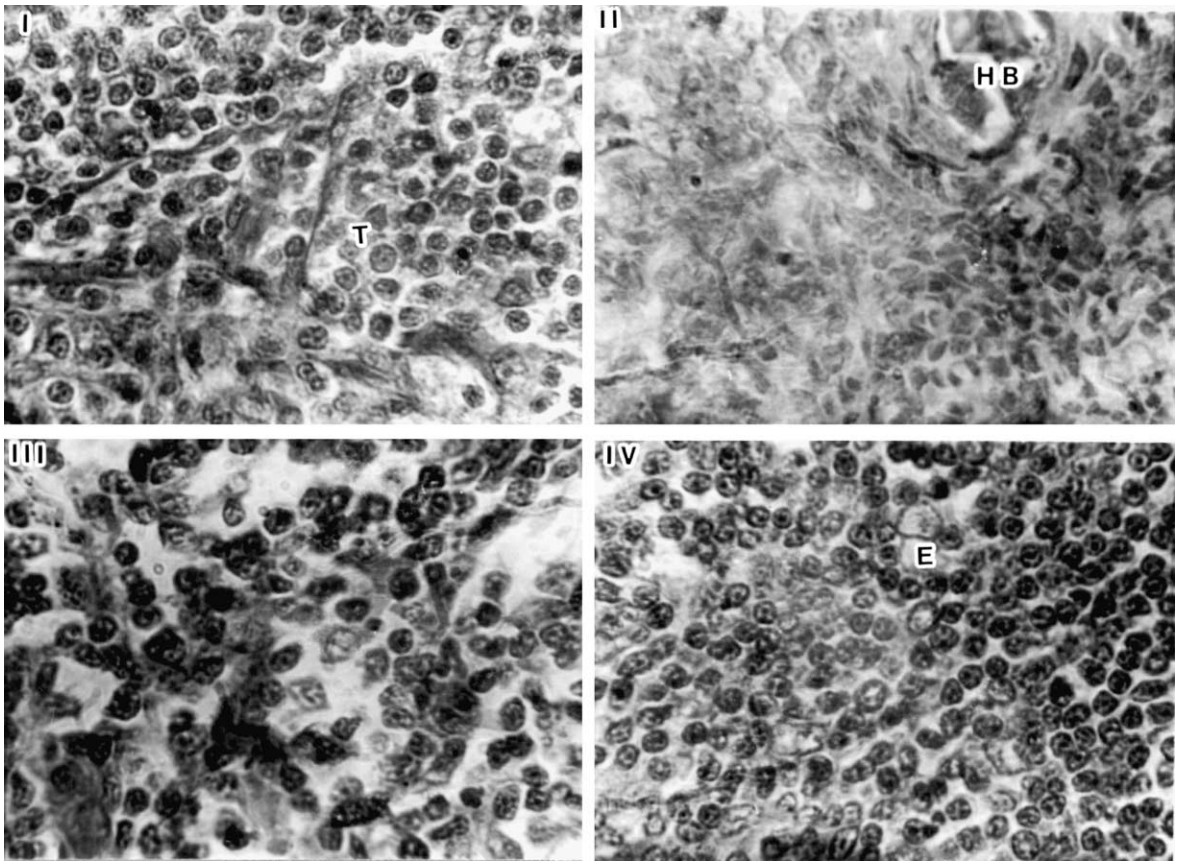

Fig. 8. (I) Histology of thymus gland of adult squirrel showing thymocytes (T) in thymic lobules during reproductive inactive phase (November to December) 365×. (II) Histology of thymus gland of adult squirrel following dexamethasone treatment showing severe depletion of thymocytes and highly pycknotic, cystic Hassal's Body (HB) in thymic lobules during reproductive inactive phase (November to December) 365×. (III) Histology of thymus gland of melatonin and dexamethasone-treated squirrel showing restoration of thymocytes in thymic lobules during reproductive inactive phase (November to December) 365×. (IV) Histology of thymus gland of melatonin-treated squirrel showing more dense thymocytes in thymic lobules with rarely seen epithelial cell (E) during reproductive inactive phase (November to December) 365×.

variation as reports suggest that cortisol treatment of ducklings reduce the number of thymic melatonin receptors [41]. Similarly, chronic melatonin treatment has been reported to decrease the density of thymic glucocorticoid receptors in rats [42].

Data on plasma melatonin hormone showed that long term (60 days) treatment of dexamethasone caused a significant decrease of plasma melatonin. However, significant restoration of plasma melatonin level was found in simultaneous dexamethasone and melatonin-treated squirrels. Studies indicate a direct effect of melatonin on immune function. The possible immunoenhancing effect of melatonin might be due to the release of cytokines and melatonin-induced immuno opiods (MIIO) by immune cells [43] whenever there is a stress-induced secretion of glucocorticoids. Further, melatonin may increase the general sensitivity of the hypothalamo–pituitary–adrenal cortical axis (HPA) to negative feedback. All these reports prove that melatonin may antagonize the dexamethasone-induced inhibition of antibody production.

Our study clearly suggests an inverse relation and feedback mechanism between pineal and adrenal of this rodent, *F. pennanti*. Variation in circulatory melatonin (high) which is known as anti-stress hormone might have played an important role in adaptation of the immune status of this seasonally breeding rodent Indian palm squirrels during reproductively inactive phase.

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References

[1] Black PH. Central nervous system—immune system interaction: psychoneuroendocrinology of stress and its immune consequences. Antimicrob Agents Chemother 1994;38:1–6.

[2] Ambrose CT. The essential role of corticosteroid in the induction of the immune response in vitro. In: Wolstenholme GEW, Knight J, editors. Hormones and immune response. London: Churchill; 1970. p. 100.

[3] Ader R, Cohen N. Phychoneuroimmunology: conditioning and stress. Ann Rev Psychol 1993;44:53–85.
[4] Besedovsky HO, Del Rey A. Feed-back interactions between immunological cells and the hypothalamus pituitary adrenal axis. Neth J Med 1991;39:274–80.
[5] Hall NR, Goldstein AL. Role of thymosin and the neuroendocrine system in the regulation of immunity. In: Fabris N, Garaci E, Hadden J, Mitchison NA, editors. Immunoregulation. New York: Plenum Press; 1983. p. 141.
[6] Maestroni GJM, Conti A, Pierpaoli W. Role of the pineal gland in immunity: circadian synthesis and release of melatonin modulates the antibody response and antagonizes the immunosuppressive effect of corticosterone. J Neuroimmunol 1986;13:19–30.
[7] Caroleo MC, Frasca D, Nistico G, Doria G. Melatonin as immunomodulator in immunodeficient mice. Immunopharmacology 1992;23:81–9.
[8] Maestroni GJM. The immunoneuroendocrine role of melatonin. J Pineal Res 1993;14:1–10.
[9] Rogers N, Vanden Heuvel C, Dawson D. Effect of melatonin and corticosteroid on in vitro cellular immune function in humans. J Pineal Res 1997;22:75–80.
[10] Singh SS, Haldar C. Effect of exogenous dexamethasone and melatonin on the immune status of Indian jungle bush quail, *Perdicula asiatica*. In: Proceedings of XX Symposium on Reproduction Biology Compared to Endocrinology, Tiruchi, January 7–9; 2002. p. 51–2.
[11] Bishnupuri KS. Maternal pineal physiology and fetal development in Indian palm squirrel *Funambulus pennanti*. Ph.D. thesis, Department of Zoology, Banaras Hindu University, Varanasi; 1999.
[12] Singh R. Pineal and melatonin control of thymus thyroid immune response of Indian palm squirrel *Funambulus pennanti*. Ph.D. thesis, Department of Zoology, Banaras Hindu University, Varanasi; 1999.
[13] Phanuphak P, Moorhead JW, Claman HN. Tolerance and contact sensitivity to DNFB in mice. In vivo detection by ear swelling and correlation with in vitro cell stimulation. J Immunol 1974;112:115–23.
[14] Attanasio A, Borrell PP, Gupta D. Circadian rhythms in serum melatonin from infancey to adolescence. J Clin Endocrinol Metab 1985;61:388–9.
[15] Mattingly DA. Simple flurometric method for the estimation of free 11-hydroxcorticoids in human plasma. J Clin Pathol 1962;15:374–9.
[16] Pauly JL, Sokal JE. A simplified technique for in vitro studies of lymphocytes activity. Proc Soc Exp Biol Med 1972;140:40–4.
[17] Haldar C, Saxena N. Ecofactors and effect of pinealectomy in regulation of seasonal testicular cycle in the Indian palm squirrel, *Funambulus pennanti*. Interdiscipl Cycle Res 1990;21:1–11.
[18] Goetzl EJ, Sreedharan SP. Mediators of communication and adaptation in the neuroendocrine and immune system. FASEB J 1992;6:2646–52.
[19] Ben-Nathan D, Kobiler D, Feuerstein G, Lustig S. Anti-stress effect of dehdroepiandrosterone (DHEA) on mice inoculated with attenuated arbovirus. Prog Neuroendocrin Immunol 1992;5:229–34.
[20] Ben-Nathan D, Maestroni GJM, Lustig S, Conti A. Protective effects of melatonin in mice infected with encephalitis viruses. Arch Virol 1995;140:223–30.
[21] Srivastava M. Role of pineal gland in regulation of reproduction in a tropical mammal the Indian palm squirrel *Funambulus pennanti*. Ph.D. thesis, Department of Zoology, Banaras Hindu University, Varanasi; 1986.
[22] Ishi T, Nakamura M, Yagi H, Soga H, Kayaba S, Gotoh T, et al. Glucocorticoid induced thymocyte death in the murine thymus: the effect at later stages. Arch Histol Cytol 1997;60:65–78.
[23] Kerr JFR, Winterford CM, Harmone BV. Apoptosis: the significance in cancer and cancer therapy. Cancer 1994;73:2013–26.
[24] Sainz RM, Mayo JC, Uria H, Kotler M, Antolin I, Roadriguez C, et al. The pineal neurohormone melatonin prevents in vivo and in vitro apoptosis in thymocytes. J Pineal Res 1995;19:178–88.
[25] Pierpaoli W, Regelson W. Pineal control of aging: effect of melatonin and pineal grafting on aging mice. Proc Natl Acad Sci USA 1994;91:787–91.
[26] Labunets IF, Butenko HM. Effect of the changes of epiphysial functions on the central and peripheral immune system in mice. Fiziol Zh 1993;39:40–6.
[27] Felsner P, Hofer D, Rinner I, Porta S, Korsatko W, Schauenstein K. Adenergic suppression of peripheral blood T-cell reactivity in the rat is due to activation of peripheral α_2-receptors. J Neuroimmunol 1995;57:7–34.
[28] Arthur WH. Histology. 3rd ed. London: Pitman Madical Publishing Co., Ltd.; 1957.
[29] Haldar C, Haussler D, Gupta D. Effect of pineal gland on circadian rhythmicity of colony forming units for granulocytes and macrophages (CFU–GM) from rat bone marrow cell-culture. J Pineal Res 1992;12:79–83.
[30] Trout JM, Mashaly MM, Siegel HS. Changes in the profiles of circulating white blood cells, corticosterone, T_3 and T_4 during the initiation of humoral immunity in immature chickens. Dev Comp Immunol 1988;12:331–46.
[31] Maestroni GJM, Conti A. The pineal neurohormone melatonin stimulates activated CD4+, Thy-1+ cells to release opiod agonist(s) with immunoenhancing and anti stress properties. J Neuroimmunol 1990;28:167–76.
[32] Maestroni GJM, Conti A. Melatonin in relation to the immune system. In: Yu HS, Reiter RJ, editors. Melatonin: biosynthesis, physiological effects and clinical application. Boca Raton: CRC Press; 1993. p. 289–311.
[33] Conti A, Maestroni GJM. Melatonin induced immuno-opioids: role in lymphoproliferative and autoimmune diseases. Adv Pineal Res 1994;7:83–100.
[34] Maestroni GJM, Flamigni LF, Hertens H, Conti A. Biochemical and functional characterization of melatonin-induced-opioids in spleen and bone marrow T-helper cells. Neuroendocrinol Lett 1995;17:145–52.
[35] Maestroni GJM, Conti A, Lissoni A. Hematopoietic rescue via T-cell dependent endogenous GM–CSF by the pineal neurohormone melatonin in tumor bearing mice. Cancer Res 1994;54:2429–32.
[36] Calvo JR, Rafil-El-Idrissi M, Pozo D, Guerrero JM. Immunomodulatory role of melatonin: specific-binding sites in human and rodent lymphoid cells. J Pineal Res 1995;18:119–26.
[37] Martin-Cacao A, Lopez-Gonzalez MA, Reiter RJ, Calvo JR, Guerrero JM. Binding of 2-[^{125}I]melatonin by rat thymus membranes during postnatal development. Immunol Lett 1993;36:59–64.
[38] Rafii-el-Idrissi M, Calvo JR, Pozo D, Harmouch A, Guerrero JM. Specific binding of 2-[^{125}I] iodomelatonin by rat splenocytes: characterization and its role on regulation of cyclic AMP production. J Neuroimmunol 1995;57:171–8.
[39] Guerrero JM, Reiter RJ. A brief survey of pineal gland—immune system interrelationships. Endocr Res 1992;18:91–113.
[40] Haldar C, Singh R. Pineal modulation of thymus and immune function in a seasonally breeding rodent *F. pennanti*. J Exp Zool 2001;297:90–8.
[41] Poon AMS, Liu ZM, Tang F, Pang SF. Cortisol decreases 2-[^{125}I] iodomelatonin binding sites in the duck thymus. Eur J Endocrinol 1994;130:320–4.
[42] Persengiev S, Patchev Y, Velev B. Steroid hormone receptors in the thymus: a site of immunomodulatory action of melatonin. Int J Biochem 1991;23:1483–5.
[43] Maestroni GJM, Conti A, Pierpaoli W. Role of the pineal gland in immunity. III. Melatonin antagonizes the immunosuppressive effect of acute stress via an opiatergic mechanism. Immunology 1988;63:465–9.