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Melatonin and differential effect of L-thyroxine on immune system of Indian tropical bird *Perdicula asiatica*

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Abstract

Interaction of thyroxine and melatonin on immune status was noted in vivo and in vitro when peripheral melatonin was high and thyroxine low in plasma of male *Perdicula asiatica* during reproductively inactive phase. During this phase exogenous thyroxine (4 μg/100 g. Bwt./day) and melatonin (25 μg/100 g. Bwt./day) increased immune parameters (spleen weight, total leukocyte count, lymphocyte count, percent stimulation ratio) and increased splenocyte density in spleen. In vitro L-thyroxine (10^{-6} M/ml) supplementation decreased the splenocyte proliferation which was reversed by melatonin (500 pg/ml) supplementation. In vivo L-thyroxine showed immunoenhancing effect while in vitro it decreased the splenocyte proliferation presenting a differential effect. In the absence of internal physiological conditions of the birds, T_4 showed a negative effect on splenocytes proliferation in vitro when treated alone. However, melatonin maintained its lymphoproliferative effect under both conditions. Thus, avian splenocyte exposed to different hormonal conditions in vitro might have produced different signal peptides other than in vivo, thereby making the result different.

Keywords: Melatonin; L-Thyroxine; Biphasic; Splenocyte; Blastogenic response; In vivo; In vitro

1. Introduction

In birds and mammals, the thyroid gland and its hormones have been reported to influence reproduction (Haldar et al., 1992; Maitra et al., 2000, 1996), metabolism (Lewinski, 1990). Thyroxine (T_4) has been reported to cause marked enlargement of the thymus and increased peripheral lymphocytes (Hassman et al., 1985) while thyroidectomy resulted in hypoplasia of lymphoid organ (Haldar and Singh, 2001) in mammals. In immunodeficient Snell bag dwarf mice thyroxine markedly increased the nucleated spleen cells, plaque-forming cells, and restored the immunological capacity of the animal (Baroni et al., 1969; Pierpaoli et al., 1969). In contrast, few authors have reported that thyroid hormones have no effects on the immune response either in vivo or in vitro (Weetman et al., 1984), while Gupta et al. (1983) reported that thyroid hormone have immune inhibitory effect in mice.

The pineal gland and its principal neurohormone melatonin affect thyroid function (Lewinski, 1990; Shavali and Haldar, 1998) and lymphatic tissue sizes (Haldar and Singh, 2001) of mammals. Virtually in all cases melatonin was examined for its effects on humoral and cell mediated immunity in mammals and never in any avian species though the pineal-thyroid interrelationship in an avian species, *Perdicula asiatica* has been examined in detail (Haldar and Ghosh, 1988) and an inverse relationship was noted. Further, there is no report available regarding the pineal and thyroid gland modulating immune function in any avian species in general and in a tropical seasonally breeding bird in particular. Therefore, in the present investigation we accessed the interaction of melatonin (which influence reproduction in birds and immunity in mammals) in relation with thyroxine (which is responsible for metabolism,

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reproduction in birds, and T cell maturation in mammals) on immune function of a tropical seasonally breeding bird, the Indian jungle bush quail, *Perdicula asiatica*.

2. Materials and methods

All the experiments were conducted in accordance with institutional practice and within the framework of revised animals (Scientific Procedures) Act of 2002 of Govt. of India on Animal Welfare.

Adult male quails were collected from the vicinity of Varanasi (Latitude 25 ° 18'N, Latitude 83 ° 01'E) during reproductively inactive phase (in the month of November). The birds were maintained in aviary exposed to ambient environmental conditions (day length 11L: 13D; temp. 13–22 °C) and were fed with millet seed (*Pennisetum typhoides*) mixed with other food grains (paddy, oat, grass seeds, and small lentils etc.) to match with diet in wild and water ad libitum for 2 weeks and then randomly divided into two sets. The nutritional stress if any, was checked by noting initial and final body weight of the birds during experiment, which was always non-significant.

Set I. Birds were divided into four groups and each groups contained seven young adult male birds. The first groups of bird were injected with normal ethanolic saline (0.01% ethanol) 0.1 mL/day. L-thyroxine (T_4, Sigma, St. Louis, USA) at a concentration 2 μg/bird/day (near physiological level dose; dissolved in few drops 0.01 N NaOH and diluted with normal saline) was subcutaneously injected to second groups of bird (Chaturvedi and Thapliyal, 1980). Melatonin (Mel, Sigma, St. Louis, USA) at a concentration of 25 μg/100 g body mass (near physiological level dose; dissolved in few drops of ethanol and diluted with normal saline) was subcutaneously injected to third groups of birds (Singh and Haldar, 2005). Fourth group of birds received both melatonin and thyroxine at 1 h interval. All the administrations were given during evening hours for 30 consecutive days. After 24 h of last injection the birds were sacrificed by decapitation during the dark phase of light/dark cycle.

2.1. Hematological parameters

Blood was taken in a WBC pipette and diluted 20 times in Turk's fluid (2.0 ml Glacial acetic acid, 0.1 g mercuric chloride, one drop Aniline, and 0.2 g Gention violet) and the white blood cells counted (no./mm3) in Neubauer's counting chamber (Spencer, USA) under the microscope. Thin film of blood was prepared and stained with Leishman's stain and differential leukocyte (lymphocyte) was counted under oil immersion lens of Leitz MPV3 microscope. Lymphocyte counts (no./mm3) was determined from the total and differential leukocyte count by using the following formula:

$$\text{Lymphocyte Count} = \frac{\text{TLC} \times \text{ Lymphocyte percentage}}{100}.$$

2.2. Histological parameters

Spleen was dissected out and fixed in Bouin's fluid for routine histological examination. Paraffin transverse sections of 5 μm thickness were cut and then stained with hematoxylin and eosin. Representative photographs of each group spleen were taken with Leitz microscope under 40× magnification.

2.3. Reagent and culture medium for blastogenic response

Tissue culture medium RPMI–1640 and all other chemicals were purchased from Sigma–Aldrich Chemicals, USA. The culture medium was supplemented with 100 μg/ml Streptomycin, 100 U/ml Penicillin, and 10% Fetal calf serum. Spleen was dissected out and processed for preparation of single cell suspensions. Number of cells was adjusted to 1×10^6 cells/ml in culture medium. Two milliliters of spleen cell suspension of each group were placed in duplicates culture tubes and kept at 37 °C in a 5% CO_2 incubator for 72 h. Blastogenic response was measured in terms of [^{3}H]thymidine (BARC, India; specific activity 8.9 Ci/mM) uptake against stimulation by Concanavalin A (Con A; T cell mitogen; SIGMA, USA) of the splenocytes (Pauly and Sokal, 1972).

$$\%\text{SR} = \frac{\text{CPM with Con A}}{\text{CPM without Con A}} \times 100$$

Set II. Twenty birds were kept in ambient environmental conditions to observe in vitro effect of L-thyroxine and melatonin. Four sets of culture were prepared with five replicas in each. First set of culture tubes were supplemented with complete culture media RPMI 1640 only. Second set of culture tubes were supplemented with medium along with melatonin (500 pg/ml). Third set of culture tubes were supplemented with medium along with L-thyroxine (10^{-6} M) and fourth set of culture tubes were supplemented with medium along with melatonin and L-thyroxine both of above concentration (Singh, 2003). Blastogenic response was measured in terms of [^{3}H] thymidine (specific activity 8.9 Ci/mM) uptake against stimulation by Con A of the splenocytes as described in Section 2.3. Reagent and culture medium for blastogenic response.

2.4. Statistical analysis

Statistical analysis of the data was performed with one way ANOVA followed by Student-Newman–Keuls' test for parametric data. The differences were considered significant when $P < 0.01$. Kruskal–Wallis test performed for non parametric data. We performed Shapiro–Wilk test to check the normality. Normality was consider when $P > 0.05$.

3. Result

3.1. Spleen weight

Melatonin treatment significantly ($P < 0.01$) increased spleen weight of birds when compared with control birds