TRADE - OFF RELATIONSHIP BETWEEN MELATONIN AND GONADAL STEROID ON MELATONIN RECEPTOR (MEL 1A R) EXPRESSION IN LYMPHOID TISSUE

Lahiri S, Rai S and Haldar C

Pineal Research Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India.

SUMMARY

Endogenous melatonin is involved in regulation of reproductive function of the photoperiodic species. A circannual rhythmicity of the pineal gland in relation to reproduction has been observed in a tropical seasonally breeding mammal, *Funambulus pennanti*. A trade-off relation is known to exist between melatonin and the gonadal steroids. *In vitro* suppression of splenocyte proliferation by testosterone and the reversal of this effect by melatonin supplementation have already been reported. Melatonin receptors have been detected in the lymphoid tissues by binding of 2-[^{125}I] iodomelatonin to splenocytes and thymocytes. We analyzed the presence of melatonin receptor adopting Western blot technique. We found that MT1 receptor protein, one of the variants of melatonin receptor, is expressed in both the thymus and spleen tissue and the expression is suppressed by testosterone treatment. This substantiates the direct immunomodulatory role of melatonin. To find the significance of steroid toxicity at molecular level, particularly testosterone, it is important to study its effect on the expression pattern of these receptors.

Key words: Melatonin, melatonin receptor, spleen, steroid, testosterone, thymus.

INTRODUCTION

The effect of gonadal steroids on immune function is best illustrated by the fact of profound sex difference in immune response (1). Males generally exhibit lower immune response than the females (1, 2). The effect of gonadal steroids on immune system was reported for the first time by Calzolari (3). A negative relationship between testosterone titer and parasite load was found in male deer (2). Further, it is seen that immune response differs according to the reproductive status of the animal. A trade-off hypothesis between the suppressive influence of gonadal steroids on immune function and an enhancing influence on breeding physiology where as melatonin has an opposite relationship was reported. Interestingly, melatonin presents a direct relationship with the immune status (4). This relationship is supported by the existence of specific melatonin binding sites in murine immunocompetent organs (5).

Melatonin mediates its action through activation of G-protein-coupled receptors (GPCRs), which have been cloned and classified as MT1 or Mel 1 a, MT2 or Mel 1b, Mel 1c and Mel 1 d (6). Initially, PCR-based strategies employing degenerate primer sequences were employed but the ultimate breakthrough came only after the sequence was determined. MT1and MT2 are found in higher vertebrates (7, 8). The mammalian melatonin receptors MT1and MT2 are 60% identical in amino acid sequence and bind 2-iodomelatonin with high affinity (7). The cloned and sequenced melatonin receptors have seven predicted trans-membrane a-helical domains. These trans-membrane domains are believed to be involved in specific interaction with ligands. The rationale to understand the interaction between any receptor and its ligands lies in the development of safe, specific therapeutic compounds to target the receptor for the benefit of human health. This is very much true for melatonin receptors located in lymphoid tissues.

Till date expression of these receptors in lymphatic tissues and its correlation with immunity has not been studied in any seasonally breeding tropical rodent. The biological significance of activity of an animal can be correlated with the level of melatonin, the expression of which is essential to act as temporal synchronizer along with other hormones for survival of the species. Hence, we studied the correlation between testosterone and melatonin on cell proliferation and receptors located in immunocompetent lymphoid tissue.

MATERIALS AND METHODS

Experimental animals

All experiments on 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. Young adult male Indian palm squirrels, *Funambulus pennanti*

Correspondence to be addressed to: Prof. Chandana Haldar, Ph.D. Email: chaldar2001@yahoo.com, chaldar@bhu.ac.in

belonging to Order Sciuridae, were collected from vicinity of Varanasi. After maintaining them in ambient environmental condition for 10-15 days (11L: 13D), they were sacrificed by decapitation. Thymus and spleen were immediately dissected out and processed for thymocyte and splenocyte culture in RPMI-1640 complete medium (9). Testostosterone [3ng/ml (10)] and melatonin [500pg/ml (11)] were supplemented in both the cultures. Four groups of cultures, each with 10 replicates, were maintained.

Group 1 Control

Group 2 Testosterone supplementation

Group 3 Testosterone + melatonin supplementation

Group 4 Melatonin supplementation

Blastogenic response

RPMI-1640 and all other chemicals were purchased from Sigma Chemical Co, St. Louis, USA. The culture medium was supplemented with 1000u/ml penicillin, 100 mg/ml streptomycin and 10% fetal calf serum. The cell number was adjusted to $1x10^6$ cells /ml. The culture tubes were placed in a CO_2 incubator (Heracell, Heraus, Germany) with 95% air: 5% CO_2 at 37^0c for 72 h. Blastogenic response was studied in terms of [^{3}H] thymidine (specific activity 8.9Ci/mM) uptake against stimulation by Con A (9).

Western blot analysis

The cells, including from bone marrow, were washed in PBS and scraped off from the culture tube into fresh centrifuge tubes, centrifuged at 770rpm for 5min. A10% homogenate was made with lysate buffer [1% NP40 (W/V), 0.1% SDS, 0.1% Aprotinin, 20 ml of 50mM sodium orthovanadate, 1% PMSF in PBS] and mixed well, centrifuged at13000rpm for 10min at 4^0C. The protein content of the suprenatant was determined using Bradfords kit (Amersham, UK). 10pg of protein was run on a 12% gel with rainbow markers (Amersham, RNP480), electrotransferred (Fastblot, Biometra) on to PVDF membrane followed by immunodetection by Mel 1 a R antibody (goat IgG, sc13179, Santacruz Biotech, USA) and detected using chemiluminescence (ECL) system (Amersham, UK). After localization of Mel 1a R, the PVDF membrane was stripped with 10% sodium azide and processed for b actin expression.

RESULT

Blastogenic response of thymocytes and splenocytes in terms of percent stimulation ratio (%SR)

Blastogenic response following testosterone supplementation showed significant (P<0.01) decrease in splenocyte and thymocyte proliferation than melatonin and melatonin + testosterone supplementation groups. Melatonin supplementation alone produced restoration of the immune status matching that in the control group (Figs. 1 a, b, 2a, b).

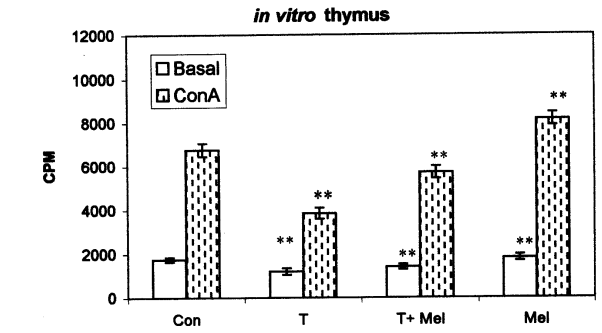

Fig. 1a

Fig. 1a. Effect of testosterone (T) and melatonin administration on basal and mitogen Con A-induced blastogenic response of thymocytes of Indian palm squirrel, *Funambulus pennanti*, during reproductively quiescent phase (Nov-Dec.). Histograms represent Mean ± SEM. Con = Control, Mel = Melatonin, T = Testosterone. ** P<0.01; Con *vs* T, Con *vs* Mel, Con *vs* Mel + T and T *vs* Mel + T.

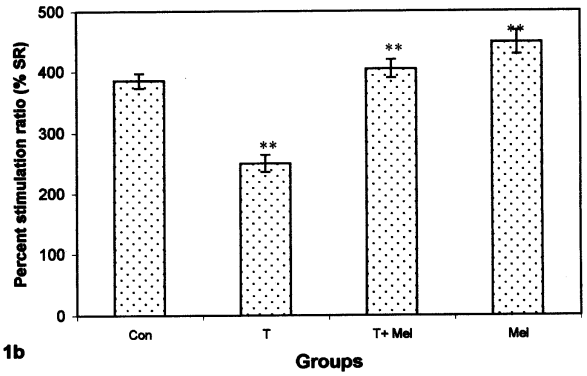

Fig. 1b

Fig. 1b. Effect of testosterone (T) and melatonin administration on % stimulation ratio (% SR) of basal and mitogen Con A-induced blastogenic response of thymocytes of Indian palm squirrel, *Funambulus pennanti*, during reproductively quiescent phase (Nov-Dec). Histograms represent Mean ± SEM. Con = Control, Mel = Melatonin, T= Testosterone. ** P<0.01; Con *vs* T, Con *vs* Mel, Con *vs* Mel + T and T *vs* Mel + T.

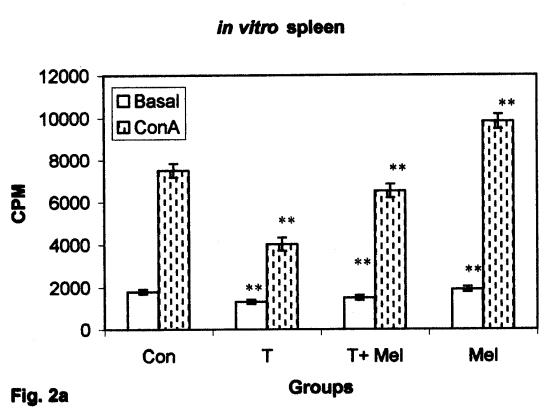

Fig. 2a. Effect of testosterone (T) and melatonin administration on basal and mitogen Con A-induced blastogenic response of splenocytes of Indian palm squirrel, *Funambulus pennanti*, during reproductively quiescent phase (Nov-Dec.). Histograms represent Mean ± SEM. Con = Control, Mel = Melatonin, T = Testosterone. ** P<0.01; Con *vs* T, Con *vs* Mel, Con *vs* Mel + T and T *vs* Mel + T.

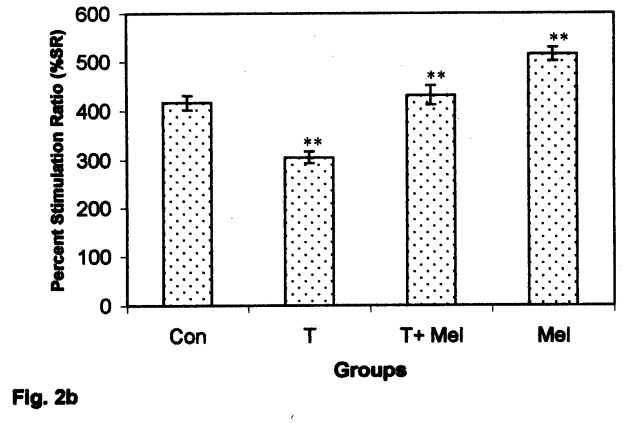

Fig. 2b. Effect of testosterone (T) and melatonin administration on % stimulation ratio (% SR) of basal and mitogen Con A-induced blastogenic response of splenocytes of Indian palm squirrel, *Funambulus pennanti*, during reproductively quiescent phase (Nov-Dec). Histograms represent Mean ± SEM. Con = Control, Mel = Melatonin, T = Testosterone. ** P<0.01; Con *vs* T, T *vs* Mel + T, Con *vs* Mel.

Western blot analysis of Mel 1a R

Testosterone supplementation down-regulated Mel 1a R expression in all lymphoid tissues i.e., spleen, thymus and bone marrow when compared with untreated control (Fig. 3a, b)

DISCUSSION

Earlier reports suggested that gonadal steroids, such as testosterone, have inhibitory influence on cell-mediated immunity (12). Our experiments were performed during reproductively inactive phase (RIP) when the peripheral testosterone level was low in order to explain the relationship between testosterone and melatonin on immune status of a seasonally breeding rodent when the environmental condition (winter) is stressful having a demand of high level of immunity. Melatonin stimulated blastogenic response while testosterone suppressed it. Combination of both suggests a dominating stimulatory effect of melatonin on proliferation of thymocytes and splenocytes that was suppressed by testosterone, or in other words recovery of suppressed proliferation rate.

The inverse relationship between testosterone and melatonin suggests that variation in peripheral melatonin level not only provides the environmental cue to the seasonally breeding animals but also regulates their immune status *via* specific melatonin receptor Mel 1a R expressed in lymphoid tissue. Mel 1a R was down-regulated by testosterone, which suggests a direct mechanism of immunosuppression by testosterone *via* Mel 1a R receptor. Interestingly, testosterone down-regulated the expression of Mel 1a R in a tissue-specific manner, i.e., bone marrow> thymus> spleen. Further, delineation of sites and mechanism of melatonin action, and variation in its receptor expression brings opportunities for accessing the therapeutic potential of this multifunctional hormone.

ACKNOWLEDGEMENT

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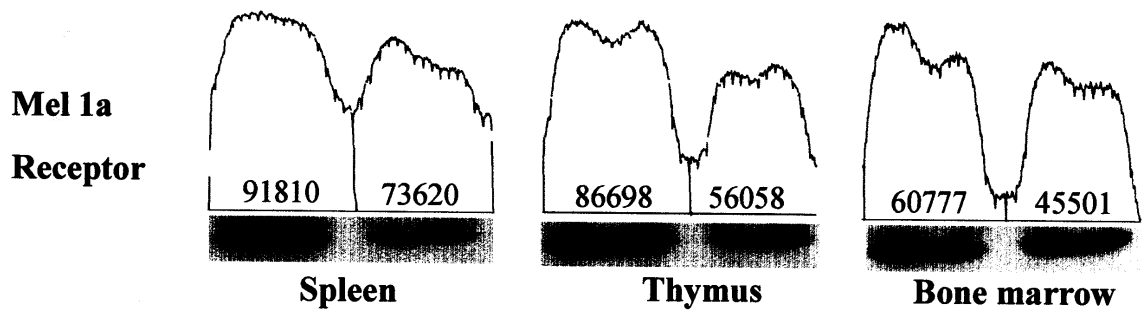

Fig. 3a

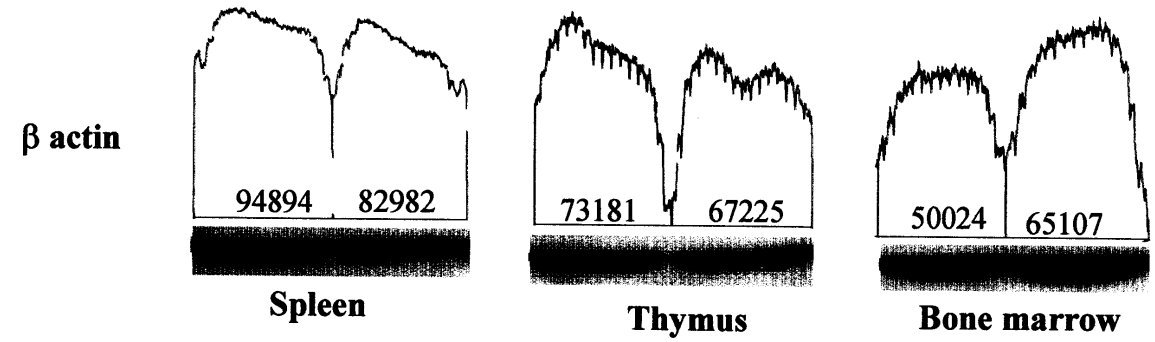

Fig. 3b

Fig.3a. Melatonin receptor (Mel 1a) expression in lymphoid tissues (spleen, thymus and bone marrow lymphocytes) following testosterone supplementation in *Funambulus pennanti*. Upper panel shows pixel values following Scion Image Analysis Software.

Fig.3b. Expression of b actin (as house - keeping) in lymphoid tissues (spleen, thymus and bone marrow lymphocytes) of *Funambulus pennanti*. Upper panel shows pixel values following Scion image analysis software.

REFERENCES

1 Schuurs AH, Verheul HAM (1990) J Steroid Biochem 35: 157-172.

2 Zuk M, Mckean KA (1996) Int J Parasitol 26: 1009-1024.

3 Calzolari A (1898) Arch Ital Biol 30: 71-77.

4 Rai S, Haldar C (2003) Comp Biochem Physiol (C: Toxicol Pharmacol) 136**:** 319-328.

5 Carrillo-Vico A, Preganeda C, Naji L, CalvoJ R, Romero MP, Guerrero JM (2003) CMLS Cell Mol Life Sc 60: 2272-2278.

6 Shiu SYW, Chow PH, Yu ZH (1996) Life Sc 59:1165.

7 Reppert SM, Weaver DR, Ebisawa T (1994) Neuron 13: 1177-1185.

8 Reppert SM, Weaver DR (1995) Cell 3: 1059-1062.

9 Pauly JL, Sokal JE (1972) Proc Soc Exp Biol Med 140: 40-44.

10 Bilbo S, Nelson RJ (2001) Am J Physiol 280: R 207-R213.

11 Drazen DL, Bilu D, Bilbo SD, Nelson RJ. 2001 Am J Physiol 280: R1476-R1482.

12 McCruden AB, Stimson WH (1991) In: Ader R, Cohen J (eds) *Psychoneuroimmunology.* pp 475-493. Academic Press, New York.