Annals of Thyroid Research

Open Access

Austin Publishing Group

Research Article

Receptor Mediated Action of Melatonin in Pituitary - Thyroid Axis of Lipopolysaccharide Challenged Mice

Laskar P and Singh SS*

Department of Zoology, Tripura University, India

***Corresponding author:** Singh SS, Department of Zoology, Tripura University, Suryamaninagar- 799 022, Tripura (W), India

Received: July 29, 2020; **Accepted:** September 02, 2020; **Published:** September 09, 2020

Abstract

Background: Lipopolysaccharide (LPS) is an endotoxic component of gram negative bacteria. Introduction of LPS causes the disruptions of many normal physiological systems including the endocrine system. The secretion of the Pineal gland, Melatonin performs as a protective component against various pathophysiological conditions through its two receptors MT1 and MT2. The aim of the present study is to focus the receptors mediated effects of melatonin in pituitary-thyroid axis in LPS challenged Swiss albino mice.

Methods: The immunohistochemical study was done to observe the localization of melatonin receptors in pituitary-thyroid axis. The western blotting analysis was performed to check the expressions of melatonin receptors in pituitary-thyroid axis as well as the expression of TSH receptors in thyroid gland. The status of pituitary-thyroid axis was observed by measuring the serum T3, T4 and TSH hormones.

Results: The significant decreases of T3, T4 and TSH hormones were noted whereas the TSH receptor of thyroid was unaltered after the intra-peritoneal injection of LPS. But administration of melatonin in LPS challenged mice was recovered the T3, T4 and TSH hormones levels with subsequent increases of TSH receptors of thyroid. Melatonin also changed the expression patterns of its receptors, MT1 and MT2 in pituitary-thyroid axis which was also affected due to LPS exposure.

Conclusion: The results may suggest the counteraction of melatonin in LPS induced distractions of pituitary-thyroid axis. It may also concludes that melatonin through mediation of its membrane bound receptors MT1 and MT2 recover the pituitary-thyroid axis of mice from LPS induced patho-physiological condition.

Keywords: Melatonin; LPS; Pituitary-thyroid axis; MT1 and MT2 receptors

Abbreviations

LPS: Lipopolysaccharide; TSH: Thyroid Stimulating Hormone; TLR-4: Toll Like Receptor-4; HPT: Hypothalamus-Pituitary-Thyroid; MT: Melatonin

Introduction

Lipopolysaccharide (LPS) is the major molecular component present in the cell wall of gram negative bacteria [1,2]. Induction of such bacterial endotoxin emerges the septic shock and creates the pathophysiological conditions including the endocrine disruptions. Administration of LPS of bovine endometrial epithelial cells causes differential DNA methylation of genes associated with inflammation and endometrial function [3]. It induces an endocrine switch from prostaglandin $F_{2\alpha}$ to prostaglandin E_2 in bovine endometrium [4] and can also modulate the concentration of gonadal hormones [5]. LPS can support the progression of tumour in the Pituitary gland in a subset of TLR-4 expressing adenomas during infectious or inflammatory processes [6]. The normal functioning of pituitary-thyroid axis may decline due to LPS exposure. Simultaneous changes were found in central and peripheral components of the Hypothalamus-Pituitary-Thyroid (HPT) axis in lipopolysaccharide induced acute illness in mice [7].

Melatonin, an indoleamine secreted from the Pineal gland is mainly involved in the regulation of circadian rhythm of mammals and other vertebrates. Besides playing an important role as a transmitter of photoperiodic information, this hormone has antioxidant [8-10], anti-aging [11,12] and anti-carcinogenic activities. Melatonin also performs as a protective component against LPS-induced septic myocardial injury [13]. Among its various physiological activities, melatonin has also associated in the regulation of secretory and growth processes of the thyroid gland [14]. In mammalian system, melatonin signals through activation of its cell membrane bound receptors, the MT1 and the MT2 [15-17]. These unique receptors showed distinct molecular structures [18], pharmacological characters [19] and chromosomal localization [20]. Receptor mediately melatonin can perform an anti-adrenergic action and provides protection against myocardial ischaemia/reperfusion injury [21]. Melatonin inhibits the LPS induced inflammation and oxidative stress in cultured mouse mammary tissue [22]. Studies are also suggesting the protective act of melatonin in cultured granulose cells from LPS-induced damage and provide evidence that melatonin might have the therapeutic utility in ovarian follicle infection in Japanese quail [23]. Although the above

Citation: Laskar P and Singh SS. Receptor Mediated Action of Melatonin in Pituitary - Thyroid Axis of Lipopolysaccharide Challenged Mice. Annals Thyroid Res. 2020; 6(2): 263-269.

studies are revealing the protection of melatonin in LPS disrupted pathophysiological condition but very scanty reports are available about its impact on LPS stimulated pituitary-thyroid axis function. Thus the present study is designed to delineate the receptors (MT1 and MT2) mediated effect of melatonin in pituitary-thyroid axis of LPS challenged mice.

Materials and Methods

All the experiments on the animals were conducted in accordance with institutional practice and within the framework of the revised Animal (Specific Procedure) Act of 2007 of Govt. of India on animal welfare. The study was approved by Institutional Animal Ethics Committee (IAEC) with ethical clearance no. TU/IAEC/2013/V/5-3.

Animal procurement and maintenance

Healthy Swiss albino mice colonies were housed at animal house in ambient laboratory conditions having temperature of 25+2°C with alternative maintenance of light/dark cycle (12L:12D). Mice were kept in groups of seven (n=7) in polycarbonate cages (43cm x 27cm x 14cm) to avoid the crowding effect and fed with mice feed and water ad libitum.

Experimental design

For observing the effects of melatonin in lipopolysaccharide challenged condition in pituitary-thyroid axis, mice were divided into four groups having 5 mice in each group as follows:

Control (Con) group: Mice of this group were received subcutaneous injection of ethanolic saline (0.01% ethanol), 0.1 ml/day for consecutive 30 days.

Melatonin (Mel) group: Mice of this group were received subcutaneous injection of melatonin (Sigma-Aldrich Chemicals, St. Louis, USA), 25µg/100g BW/day for consecutive 30 days at evening (16:30-17:00) hours.

Lipopolysaccharide (LPS) group: Mice of this group were received single intra-peritoneal injection of Lipopolysaccharide (LPS) (Sigma-Aldrich Chemicals, St. Louis, USA), 250µg/100g BW. Experimental mice were sacrificed after 4 days of LPS administration [24].

Lipopolysaccharide + Melatonin (LPS + Mel) group: Mice of this group were received both LPS and melatonin. LPS was injected 4 days prior to the completion of melatonin treatment.

Sample collection and processing

After 24 hours of last administration, experimental mice were sacrificed under anaesthesia (pentobarbital, 15mg/Kg, intraperitoneal injection). Trunk blood was collected in heparinized tube. Blood serum was separated and stored at -20°C till hormones analyses. Experimental tissues (pituitary and thyroid gland) were dissected out immediately and stored at --20°C for western blot analysis. The half part of each experimental tissue was immediately fixed in Bouin's fixative for immunohistochemical localization.

Hormonal analysis

Serum T_3, T_4 and TSH hormone analyses were done by commercial ELISA Kits (Diagnostic AutomatationInc, CA, USA). For T_3, detection range 0-10ng/mL, specificity 96.30% and sensitivity was 0.2ng/mL. For T_4, detection range 0-30µg/dL, specificity 96.30% and sensitivity was 0.05µg/mL. For TSH, detection range 0-40µIU/mL, specificity 100% and sensitivity was 0.20µIU/mL.

Immunohistochemical staining

Immunohistochemical staining was done to observing the melatonin receptors localization [25] in pituitary and thyroid glands. Paraffin sections (5µm) fixed on 3% gelatine coated slides were deparaffinised and rehydrated with alcohol grades. The sections were placed in PBS for 30 minutes and endogenous peroxide activity was blocked by 0.3% H_2O_2 in methanol for 30 minutes at room temperature (25°C). Sections were washed thrice with phosphate buffered saline (PBS: 0.1M Na_2HPO_4, NaH_2PO_4, 0.9% NaCl, pH=7.4) and were placed in blocking solution (horse blocking serum, diluted 1:200 in PBS, PK -6200, Vector Laboratories, Burlingame, CA) for 2 hrs. Sections were incubated with primary antibodies [Mel1AR (MT1); sc13186 and Mel1BR (MT2); sc13177, goat polyclonal, Santacruz Biotech, USA, diluted 1:200] overnight at 4°C. Next day, sections were washed thrice with PBS and incubated with biotinylated secondary antibody (Vectastain ABC Universal Kit, PK-6200, Vector Laboratories, Burlingame, CA, dilution 1:1000). Sections were again washed thrice with PBS and incubated with preformed AB (Avidin-Biotin) reagent for 30 minutes. The antigens were visualized using the 0.03% peroxidase substrate 3,3′-diaminobenzidine (DAB; Sigma-Aldrich Chemicals, St. Louis, USA) in 0.01M Tris-Cl (pH=7.6) and 0.1% H_2O_2 and counterstained with Ehrlich's haematoxylin. The sections were dehydrated and mounted with DPX. Microphotographs of the stained sections were taken under 40X objective of Olympus microscope BX 41. To test the specificity of the used antibodies, the primary antibodies were not added in control sections which were treated as negative control and incubated with same dilution of normal serum for overnight at 4°C. Next morning the immunohistochemical protocol was followed under the same conditions.

Western blot analysis

Tissue samples were homogenized and lysed in RIPA buffer [1% (v/v) NP-40, 0.1% w/v] Sodium Dodecyl Sulphate (SDS) in PBS containing aprotinin, sodium orthovanadate and Phenyl Methyl Sulphonyl Fluoride (PMSF) and total sample protein was quantified by Lowry method (1951). Aliquots containing 100µg proteins were resolved by 10% (w/v) SDS polyacrylamide gel electrophoresis followed by electro transfer to nitrocellulose membrane (Santa Cruz Biotech, USA). Immune detection was carried out by using primary antibodies [Mel1AR (MT1); sc13186, Mel1BR (MT2); sc13177 and TSH-R; sc-7818, goat polyclonal, Santacruz Biotech, USA, diluted 1:100] and β-actin antibody (sc-130656, rabbit polyclonal, Santacruz Biotech, USA, diluted 1:500) diluted in PBS contained 5% skimmed milk and 0.01% Tween-20 followed by incubation with horse-radish peroxidase conjugated secondary antibodies (goat anti-rabbit IgG for β-actin antisera; diluted 1:1000 and rabbit anti-goat IgG for Mel 1AR (MT1), Mel 1BR (MT2) and TSH-R antisera; diluted 1:1000). The immune interactions were detected by using Super Signal West Pico Chemiluminescent Substrate (#34080, Thermo Scientific, Rockford, USA). Bands were quantified by measurement of optical density using Scion Image Analysis Software (Scion Corporation, MD, USA). Values were expressed as ratio of the density of the specific signal to β-actin signal and expressed as the % control value [26]. Each sample corresponds to tissue from a single animal and at least five

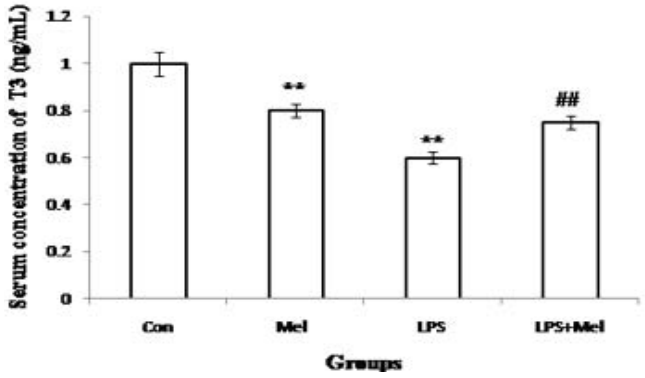

Figure 1: Serum T3 concentration in experimental groups of mice. Histogram represents Mean ± SEM. The differences were considered significant when p< 0.05. **p<0.01: Con vs Mel, Con vs LPS; ## p<0.01: LPS vs LPS+Mel.

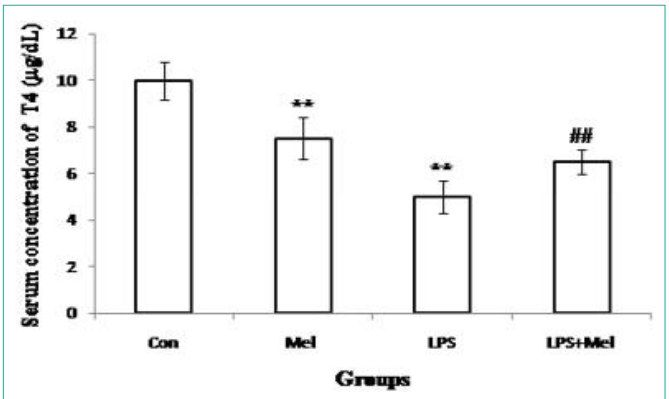

Figure 2: Serum T4 concentration in experimental groups of mice. Histogram represents Mean ± SEM. The differences were considered significant when p< 0.05. **p<0.01: Con vs Mel, Con vs LPS; ## p<0.01: LPS vs LPS+Mel.

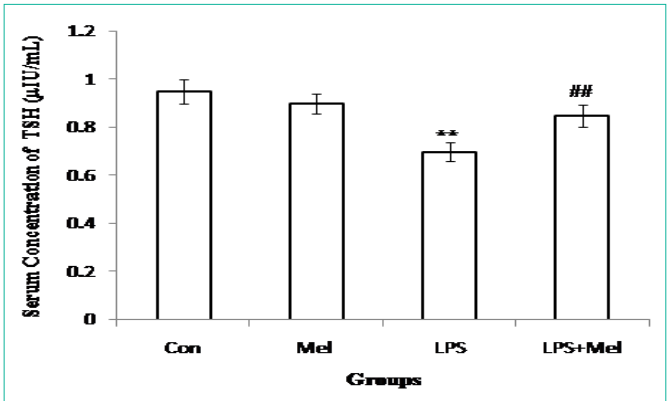

Figure 3: Serum TSH concentration in experimental groups of mice. Histogram represents Mean ± SEM. The differences were considered significant when p< 0.05. **p<0.01: Con vs LPS; ## p<0.01: LPS vs LPS+Mel.

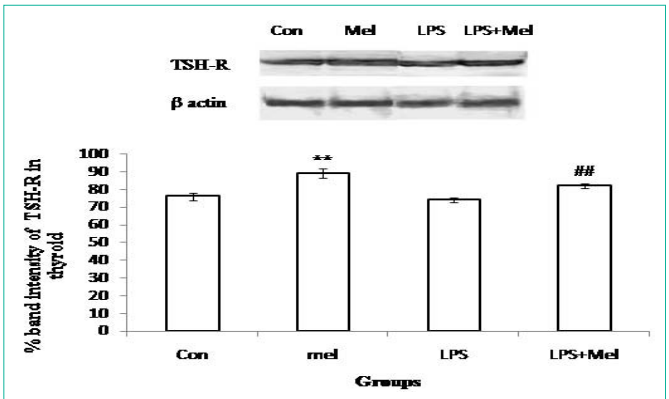

Figure 4: Western blot analysis of TSH receptors protein expression in thyroid gland. β-actin was used as loading control. Lower panel shows percent expression of protein following Scion Image analysis. Histogram represents Mean ± SEM. The mean differences were considered significant when p< 0.05. **p<0.01: Con vs Mel; ## p<0.01: LPS vs LPS+Mel.

gels corresponding to each subunit and experimental conditions were analyzed.

Statistical analysis

Statistical analysis of the data was performed with one way Analysis Of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) multiple range test. The differences were considered significant when p< 0.05. Microsoft Excel program and Statistical Package for the Social Sciences (SPSS) were used for calculation and graph preparation.

Results

Serum T3 Level

T3 hormone level was significantly (p<0.01) decreased in both melatonin treated and LPS treated group of mice in comparison with control mice. Melatonin along with LPS treatment significantly (p<0.01) increased the T3 hormone level in comparison with LPS treated group of mice (Figure 1).

Serum T4 Level

T4 hormone level was significantly (p<0.01) decreased in melatonin treated group as well as in LPS treated group of mice in comparison with control mice whereas in (LPS + Mel) group, T4 hormone level was significantly (p<0.01) increased in comparison with LPS treated group of mice (Figure 2).

Serum TSH Level

TSH hormone level was unchanged in melatonin treated group of mice and was significantly (p<0.01) decreased in LPS treatment received mice in comparison to control mice. But in (LPS+Mel) group, TSH level was increased significantly (p<0.01) in compared with single dose LPS received mice (Figure 3).

TSH-R proteins expression in thyroid gland

TSH receptor proteins expression was significantly (p<0.01) increased in melatonin treated group of mice and was unaffected in LPS treatment in compare to control group of mice. But in (LPS + Mel) group, TSH-R expression was found increased significantly (p<0.01) as compare to LPS received mice group (Figure 4).

Immunohistochemical observation of MT1 and MT2 receptors in pituitary and thyroid

Both MT1 and MT2 receptors were localized in the follicular and parafollicular cells of the thyroid gland (Figure 5). In pituitary gland, these receptors immunoreactivity were observed in pars distalis region as shown (Figure 6).

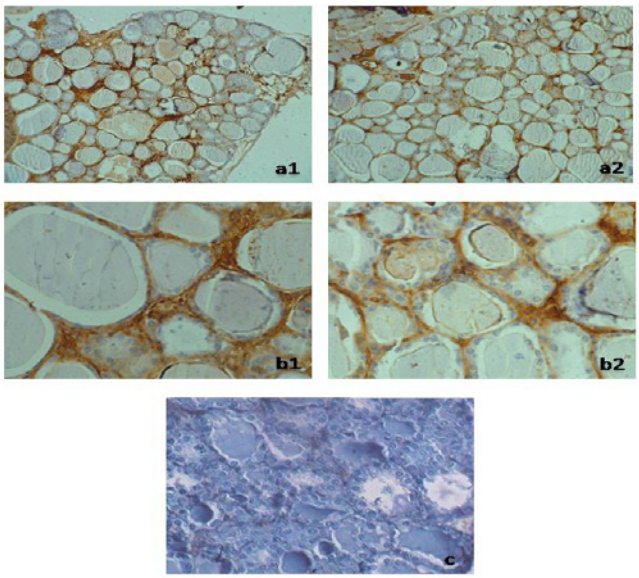

Figure 5: Immunohistochemical staining of melatonin receptors, MT1 receptors (a1: 10X magnification; b1: 40X magnification) and MT2 receptors (a2: 10X magnification; b2: 40X magnification) in thyroid glands of mice. DAB reaction was not detected in negative control section (c). Microphotographs were taken by Olympus Microscope.

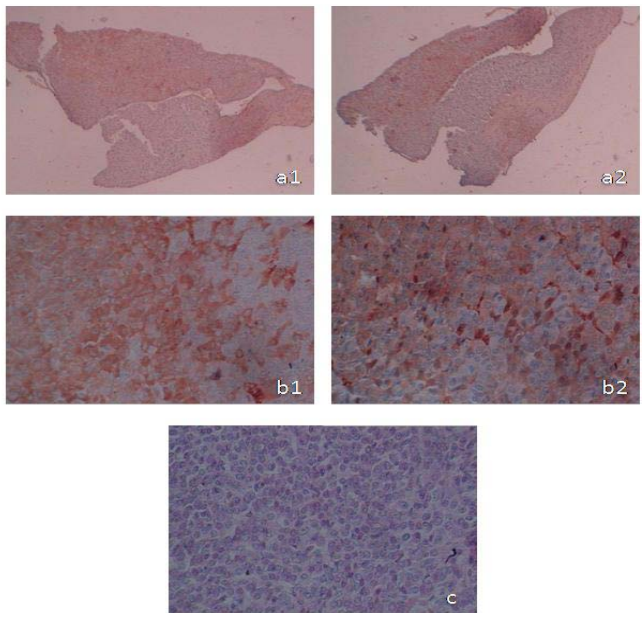

Figure 6: Immunohistochemical staining melatonin receptors, MT1 receptors (a1: 10X magnification; b1: 40X magnification) and MT2 receptors (a2: 10X magnification; b2: 40X magnification) in pituitary glands of mice. DAB reaction was not detected in negative control section (c). Microphotographs were taken by Olympus Microscope.

MT1 receptor proteins expression in thyroid gland

The expression of MT1 receptor was decreased significantly ($p<0.01$) in melatonin treated mice and was increased significantly ($p<0.01$) in LPS treated mice in comparison to control group of mice. There was significant decrease of MT1 receptor observed in (LPS+Mel) treated mice in compare to LPS treated mice (Figure 7).

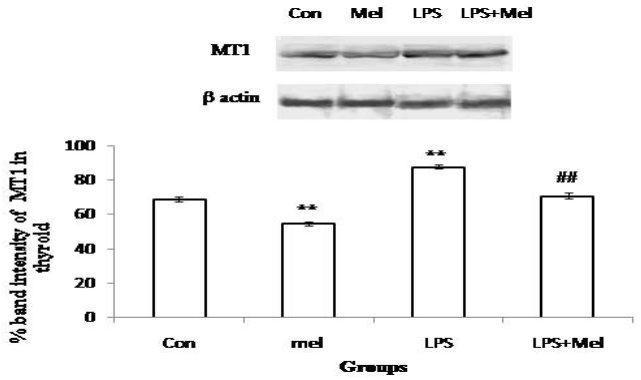

Figure 7: Western blot analysis of MT1 receptors protein expression in thyroid gland. β-actin was used as loading control. Lower panel shows percent expression of protein following Scion Image analysis. Histogram represents Mean ± SEM. The mean differences were considered significant when $p< 0.05$. **$p<0.01$: Con vs Mel, Con vs LPS; ## $p<0.01$: LPS vs LPS+Mel.

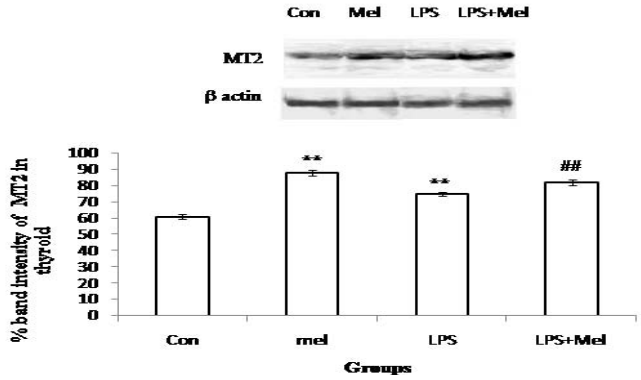

Figure 8: Western blot analysis of MT2 receptors protein expression in thyroid gland. β-actin was used as loading control. Lower panel shows percent expression of protein following Scion Image analysis. Histogram represents Mean ± SEM. The mean differences were considered significant when $p< 0.05$. **$p<0.01$: Con vs Mel, Con vs LPS; ## $p<0.01$: LPS vs LPS+Mel.

MT2 receptor proteins expression in thyroid gland

MT2 receptor protein was significantly ($p<0.01$) increased of each melatonin and LPS received mice group as compare to control mice. In (LPS + Mel) group, MT2 was increased significantly ($p<0.01$) in comparison with LPS group of mice (Figure 8).

MT1 receptor proteins expression in Pituitary gland

MT1 receptor was significantly ($p<0.01$) increased in melatonin treated mice and was unaltered in LPS treated mice in compare to control mice. Whereas, in (LPS + Mel) treated group, it was significantly ($p<0.01$) enhanced as compare to LPS received mice (Figure 9).

MT2 receptor proteins expression in Pituitary gland

Significant ($p<0.01$) increase in MT2 receptor expression was noted in melatonin treated group and was significantly ($p<0.01$) decreased in LPS treated group in comparison with control group. But MT2 expression was found to increased significantly ($p<0.01$) in (LPS + Mel) group as comparison with LPS treated group of mice (Figure 10).

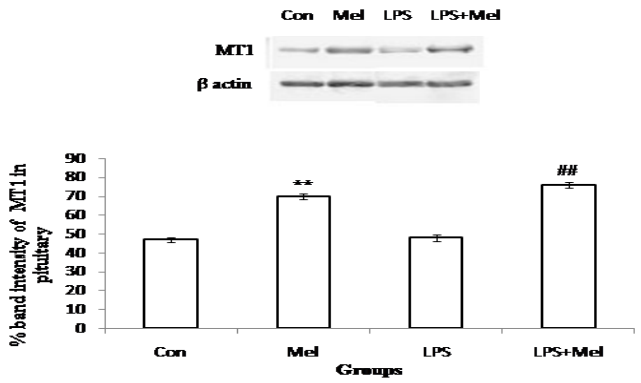

Figure 9: Western blot analysis of MT1 receptor protein expression in pituitary gland. β-actin was used as loading control. Lower panel shows percent expression of protein following Scion Image analysis. Histogram represents Mean ± SEM. The mean differences were considered significant when p< 0.05. **p<0.01: Con vs Mel; ## p<0.01: LPS vs LPS+Mel.

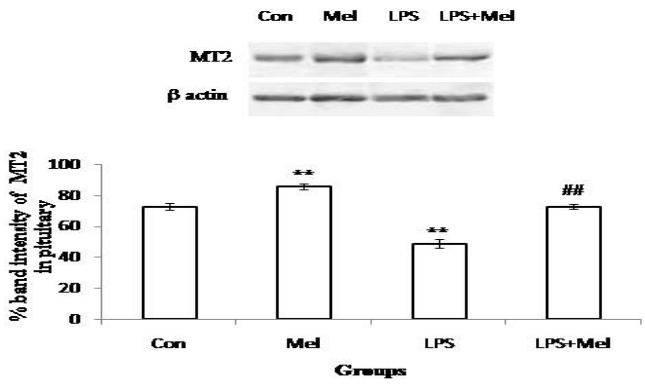

Figure 10: Western blot analysis of MT2 receptor protein expression in pituitary gland. β-actin was used as loading control. Lower panel shows percent expression of protein following Scion Image analysis. Histogram represents Mean ± SEM. The mean differences were considered significant when p< 0.05. **p<0.01: Con vs Mel, Con vs LPS; ## p<0.01: LPS vs LPS+Mel.

Discussion

Homeostatic equilibrium depends on dynamic inter-relationships between thyroid hormones and pituitary TSH [27]. The interrelationship between melatonin and thyroid gland is also well established. The pituitary-thyroid axis and melatonin interdependency status was also studied by the researchers [28]. Generally melatonin has the inhibitory effects on thyroid gland according to number of reports. In this study, we also observed the depletion of thyroid hormones levels after introducing the melatonin. A similar kind of report is available which depicted the decreasing levels of thyroid hormones in hamsters treated with melatonin [29]. Melatonin treatment suppressed the mitotic activity and thus strongly inhibited the thyroid function [30,31]. *In vitro* application of melatonin suppressed the thyroid function in dose dependent manner [32,33]. Melatonin supplementation caused increased expression of TSH receptor proteins in thyroid tissues but TSH hormone level was remaining unchanged in the studied mice. Melatonin treatment could not alter the TSH hormone levels that also reported by the investigators [34].

Endotoxic Lipopolysaccharide (LPS) is a powerful mediator of systemic inflammation and a driver of septic shock which can affect the endocrine system. In this study T3 and T4 hormone levels were noted declined due to LPS exposure. LPS caused infection might be associated with low levels of thyroid hormones. Available reports were also showed the similar decreasing pattern of serum thyroid hormones levels after the treatment of LPS [35,36]. LPS treatment also decreased the TSH level in circulation whereas TSH receptor proteins expression was unaffected on thyroid tissues. Suppression of TSH hormone concentration after LPS treatment might be due to suppression of TRH production and pituitary stimulation. Report suggested that LPS treatment caused suppression of TRH gene expression in Paraventricular Nucleus (PVN) in Non-Thyroidal Illness (NTI) patients [37].

Melatonin administration was encountered the LPS induced suppression of circulatory T3, T4 hormones levels. The thyroid pathological condition due to LPS might be counteracted through melatonin. The probable beneficial action of melatonin in autoimmune thyroid disease like pathological condition was also studied by the investigators [38]. In the present study, it was observed that melatonin treatment increased the TSH hormone that was depleted due to LPS induction. Exogenous melatonin encounters the LPS induced suppression of TRH gene expression in PVN and normalizes the TSH production from pituitary [39]. Administration of melatonin also caused the elevation of TSH-R proteins in thyroid. Melatonin may be recovered the LPS induced pituitary-thyroid axis hormonal distraction through up regulation of TSH receptor proteins of thyroid.

Immunohistochemical staining showed the localization of MT1 and MT2 melatonin receptors in the pituitary and thyroid glands of studied mice. Immunoreactivity of melatonin receptors (MT1 and MT2) were observed in the pars distalis of anterior pituitary gland. Cell specific expression of MT1 mRNA in pars distalis of embryonic pituitary in rats was also reported [40]. Various studies suggested presence of (I^{125}) iodo-melatonin binding sited in anterior pituitary [41,42]. Further, MT1 and MT2 melatonin receptors immunoreactivity were observed in follicular and parafollicular cells of thyroid gland of mice. Melatonin receptors immunoreactivity in follicular and parafollicular cells in thyroid gland of rat was also reported [43].

It was also noted through this study that administration of melatonin decreases the MT1 melatonin receptor expression and increases the MT2 receptor expression in thyroid gland. Our previous report suggested that exogenous melatonin differentially modulates the MT1 and MT2 receptor expression in thyroid gland in age dependent manner [44]. LPS treatment induced the increased expression of MT1 and MT2 receptors proteins in thyroid gland. But in melatonin along with LPS treated mice, MT1 receptor expression was decreased and MT2 receptor expression was increased in thyroid as compare to LPS group. So, melatonin might be affected on thyroid through activation of its MT2 receptors as well as by down regulation of MT1 receptors present in thyroid gland in LPS challenged condition. Our previous study also reported the MT1 and MT2 receptors mediated modulation of melatonin in thyroid gland in hyperthyroid condition of mice [45].

Increased expression of MT1 and MT2 melatonin receptor proteins were noted in pituitary gland in melatonin treated group. Melatonin treatment caused the up regulation of melatonin receptors in pituitary gland. LPS treatment to mice decreased the MT2 receptor protein whereas MT1 receptor expression was unaffected. Melatonin supplementation along with LPS treatment increased the MT1 and MT2 receptor expression in pituitary. Melatonin might be ameliorated the pathological effects of LPS through modulation of its both MT1 and MT2 receptors in pituitary gland.

Conclusion

Melatonin recovers the LPS caused damages in the tissues. In this study, LPS treatment caused suppression of thyroid hormones level by decreasing TSH secretion from pituitary. Melatonin influences on thyroid hormones by increasing the TSH hormone secreted from pituitary gland as well as by up regulation of TSH-R proteins of thyroid gland in LPS challenged mice. Further, exogenous melatonin also caused up-regulation of MT1 and MT2 receptor proteins in pars distalis of pituitary gland and MT2 receptor protein in follicular as well as parafollicular cells of thyroid gland in LPS challenged mice. Therefore, the above finding may suggest that melatonin may recover the LPS induced distraction of pituitary-thyroid axis through mediation of its receptors. In addition, further studies are necessary to elucidate the cellular and molecular mechanisms of melatonin and the contribution of its receptors (MT1 and MT2) in this aspect.

Acknowledgment

We would like to thank University Grants Commission (UGC) and Council of Scientific and Industrial Research (CSIR) for their financial support. The authors are also grateful to DBT sponsored State Biotech Hub, Tripura University for providing their laboratory equipment for this research work.

References

1. Alexander C, Rietschel ET. Bacterial lipopolysaccharides and innate immunity. J Endotoxin Res. 2001; 7: 167-202.
2. Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. Annu Rev Biochem. 2002; 71: 635-700.
3. Jhamat N, Niazi A, Guo Y, Chanrot M, Ivanova E, Kelsey G, et al. LPS-treatment of bovine endometrial epithelial cells causes differential DNA methylation of genes associated with inflammation and endometrial function. BMC Genom. 2020; 21: 385-395.
4. Herath S, Lilly ST, Fischer DP, Williams EJ, Dobson H, Bryant CE, et al. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2α to prostaglandin E2 in bovine endometrium. Endocrinology. 2009; 150: 1912-1920.
5. Ferranti EM, Aloqaily BH, Gifford CA, Loest CA, Wenzel JC, Hernandez Gifford JA. Lipopolysaccharide modulation of ovarian hormonal profile1. Transl Anim Sci. 2018; 2: 31-34.
6. Tichomirova M, Schaaf L, Feirer M, Stummer W, , Losa M, Lange M, et al. Is there a functional role of bacterial endotoxin receptor (Tlr4) in pituitary tumor progression? Exp Clin Endocrinol Diabetes. 2003; 111: 121-124.
7. Boelen A. Kwakkel J, Thijssen-Timmer DC, Alkemade A, Fliers E, Wiersinga WM, et al. Simultaneous changes in central and peripheral components of the hypothalamus-pituitary-thyroid axis in lipopolysaccharide-induced acute illness in mice. J Endocrinol. 2004; 182: 315–323.
8. Martinez-Cruz F, Pozo D, Osuna C, Espinar A, Marchante C, Guerrero JM. Oxidative stress induced by phenylketonuria in the rat: Prevention by melatonin, vitamin E, and vitamin C. J Neurosci Res. 2002; 69: 550-558.
9. Reiter RJ, Tan DX, Maldonado MD. Melatonin as an antioxidant: physiology versus pharmacology. J Pineal Res. 2005; 39: 215-216.
10. Mogulkoc R, Baltaci AK, Oztekin E, Aydin L, Sivrikaya A. Melatonin prevents oxidant damage in various tissues of rats with hyperthyroidism. Life Sci. 2006; 79: 311-315.
11. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Lopez-Burillo S. Melatonin, longevity and health in the aged: an assessment. Free Radic Res. 2002; 36: 1323-1329.
12. Tajes M, Gutierrez-Cuesta J, Ortuno-Sahagun D, Camins A, Pallas M. Anti-aging properties of melatonin in an in vitro murine senescence model: involvement of the sirtuin 1 pathway. J Pineal Res. 2009; 47: 228-237.
13. Di S, Wang Z, Hu W, Yan X, Ma Z, Li X, et al. The protective effects of melatonin against LPS induced septic myocardial injury: A potential role of AMPK-mediated autophagy. Front Endocrinol. 2020; 11: 162.
14. Lewinski A, Karbownik M. Melatonin and the thyroid gland. Neuro Endocrinol Lett. 2002; 23: 73-78.
15. Dubocovich ML, Cardinali DP, Delagrange PRM, Krause DN, Strosberg D, Sugden D, et al. Melatonin Receptors. Girdlestone D editor. In: The IUPHAR: Compendium of Receptor Characterization and Classification. IUPHAR Media. 2000; 270-277.
16. Dubocovich ML, Rivera-Bermudez MA, Gerdin MJ, Masana MI. Molecular pharmacology, regulation and function of mammalian melatonin receptors. Front Biosci. 2003; 8: 1093-1108.
17. Masana MI, Dubocovich ML. Melatonin receptor signalling: finding the path through the dark. Sci STKE. 2001; 107: 39-40.
18. Reppert SM, Weaver DR, Godson C. Melatonin receptors step into the light: cloning and classification of subtypes. Trends Pharmacol Sci. 1996; 17: 100-102.
19. Dubocovich ML, Masana MI, Iacob S, Sauri DM. Melatonin receptor antagonists that differentiate between the human Mel1a and Mel1b recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML1 presynaptic heteroreceptor. Naunyn Schmiedebergs Arch Pharmacol. 1997; 355: 365-375.
20. Slaugenhaupt SA, Roca AL, Liebert CB, Altherr MR, Gusella JF, Reppert SM. Mapping of the gene for the Mel1a-melatonin receptor to human chromosome 4 (MTNR1A) and mouse chromosome 8 (Mtnr1a). Genomics. 1995; 27: 355-357.
21. Melatonin receptor-mediated protection against myocardial ischaemia/reperfusion injury: role of its anti-adrenergic actions. J Pineal Res. 2008; 45: 449–458.
22. Yu GN, Tan W. Melatonin Inhibits Lipopolysaccharide-Induced Inflammation and Oxidative Stress in Cultured Mouse Mammary Tissue. Mediators Inflamm. 2019; 8597159.
23. Yu GM, Isobe N, Maeda T. Protective Effect of Melatonin on LPS-stimulated Granulosa Cells in Japanese Quail. J Poult Sci. 2017; 54: 319-325.
24. Zhang YH, Takahashi K, Jiang GZ, Zhang XM, Kawai M, Fukada M, et al. *In vivo* production of heat shock protein in mouse peritoneal macrophages by administration of lipopolysaccharide. Infect Immun. 1994; 62: 4140-4144.
25. Savaskan E, Wirz-Justice A, Olivieri G, Pache M, Krauchi K, Brydon L, et al. Distribution of melatonin MT1 receptor immunoreactivity in human retina. J Histochem Cytochem. 2002; 50: 519-526.
26. Treeck O, Halder C, Ortmann O. Antiestrogens modulate MT1 melatonin receptor expression in breast and ovarian cancer cell lines. Oncol Rep. 2006; 15: 231-235.
27. Hoermann R, Midgley JEM, Larisch R, Dietrich JW. Homeostatic Control of the Thyroid–Pituitary Axis: Perspectives for Diagnosis and Treatment. Front Endocrinol. 2015; 6: 1-17.
28. Bellastella A, Sinisi AA, Criscuolo T, Bellls AD, Carella C, Lorio S, et al. Melatonin and the pituitary thyroid axis status in blind adults: a possible resetting after puberty. Clin Endocrinol. 1995; 43: 707-711.

29. Champney TH. Reductions in hamster serum thyroxine levels by melatonin are not altered by changes in serum testosterone. Gen Comp Endocrinol. 2001; 2: 121-126.

30. Wajs E, Lewinski A. Inhibitory influence of late afternoon melatonin injections and the counter-inhibitory action of melatonin-containing pellets on thyroid growth process in male Wistar rats: comparison with effects of other indole substances. J Pineal Res. 1992; 13: 158-166.

31. Sewerynek E, Wiktorska J, Lewinski A. Effects of melatonin on the oxidative stress induced by thyrotoxicosis in rats. Neuro Endocrinol Lett. 1999; 20: 157-161.

32. Wright ML, Pikula A, Cykowski LJ, Kuliga K. Effect of melatonin on the anuran thyroid gland: follicle cell proliferation, morphometry, and subsequent thyroid hormone secretion in vitro after melatonin treatment *in vivo.* Gen Comp Endocrinol. 1996; 103: 182-191.

33. Wright ML, Pikula A, Babski AM, Labieniec KE, Wolan RB. Effect of melatonin on the response of the thyroid to thyrotropin stimulation *in vitro.* Gen Comp Endocrinol. 1997; 108: 298-305.

34. Siegrist C, Benedetti C, Orlando A. Lack of changes in serum prolactin, FSH, TSH, and estradiol after melatonin treatment in doses that improve sleep and reduce benzodiazepine consumption in sleep-disturbed, middle-aged, and elderly patients. J Pineal Res. 2001; 30: 34-42.

35. Yaglova NV. Relationship between functional activity of the thyroid gland and levels of proinflammatory and immunoregulatory cytokines in acute experimental endotoxicosis. B Exp Biol Med. 2009; 147: 635.

36. Castro I, Quisenberry L, Calvo RM, Obregon MJ, Lado-Abeal J. Septic shock non-thyroidal illness syndrome causes hypothyroidism and conditions for reduced sensitivity to thyroid hormone. J Mol Endocrinol. 2013; 50: 255-266.

37. Fliers E, Guldenaar SE, Wiersinga WM, Swaab DF. Decreased hypothalamic thyrotropin-releasing hormone gene expression in patients with non-thyroidal illness. J Clin Endocrinol Metab. 1997; 82: 4032-4036.

38. Angelo GD, Marseglia L, Manti S, Colavita L, Cuppari C, Impellizzeri P, et al. Atopy and autoimmune thyroid diseases: melatonin can be useful? Ital J Pediatr. 2016; 42: 95.

39. Wong CS, Jow GM, Kaizaki A, Fan LW, Tien LT. Melatonin ameliorates brain injury induced by systemic lipopolysaccharide in neonatal rats. Neuroscience. 2014: 267; 147-156.

40. Johnston JD, Klosen P, Barrett P, Hazlerigg PG. Regulation of MT1 melatonin receptor expression in the foetal pituitary. J Neuroendocrinol. 2006; 18: 50-56.

41. Heiliwell RJ, Williams LM. Melatonin binding sites in the ovine brain and pituitary: characterization during the oestrous cycle. J Neuroendocrinol. 1992; 4: 287-294.

42. Williams LM, Hannah LT, Hastings MH, Maywood ES. Melatonin receptors in the rat brain and pituitary. J Pineal Res. 1995; 19: 173-177.

43. Garcia-Marin R, Miguel De M, Fernandez-Santos JM, Carillo-Vico A, Utrilla JC, Morillo-Bernal J. et al. Melatonin synthesizing enzymes and melatonin receptor in rat thyroid cells. Histol Histopathol. 2012; 27: 1429-1438.

44. Laskar P, Acharjee S, Singh SS. Effect of exogenous melatonin on thyroxine (T4), thyrotropin (TSH) hormone levels and expression patterns of melatonin receptor (MT1 and MT2) proteins on thyroid gland during different age groups of male and female swiss albino mice. Adv Biores. 2015; 6: 7-14.

45. Laskar P, Singh SS. Melatonin modulates thyroid hormones and splenocytes proliferation through mediation of its MT1 and MT2 receptors in hyperthyroid mice. Proc Zool Soc. 2018; 71: 186-193.