Original Article

Ultra-Structural Changes of The Pineal and Adrenal Gland Under Effects of Photoperiod, Melatonin and Testosterone Propionate in The Adult Male Pigeon

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Abstract

The environmental factors, photoperiod and various experimental condition such as melatonin (MEL) and testosterone propionate (TP) hormone treatment play an important role in regulating the physiological activity in adult male domestic pigeons. Exposed constant photoperiod (24L: 0D) with exogenous MEL and TP hormone treatment at a daily dose of 20 µg/ 100gm body weight and at a daily dose of 1 mg/ 100gm body weight for 15 days treatment respectively in breeding and non breeding seasons. With constant illumination (24L : 0D) and melatonin treatment for 15 days in primary breeding season, the experimental birds exhibited adreno-cortical activity such as the number of mitochondria and lipid granuoles were increased and lysosomes. Golgi complex and smooth endoplasmic reticulum were decreased than the control birds. Where pinealocytes were decreased minimally and the Golgi complex showed the typical dictyosomal structure. Mitochondria were oval and long in shape and no remarkable changes were noticed in the number of mitochondria. Numerous free ribosomes were observed in all the studied areas. Under continuous darkness (0L : 24D) with same amount treatment of melatonin in regressive phase-I, ultra-structural features of adrenal and pineal gland were more or less similar like the control birds with melatonin treated groups. On the other hand, constant illumination and with induction of testosterone propionate (24L : 0D + TP) for 15 days in primary breeding birds, within the adrenal gland, the numbers of mitochondria were decreased. Some dead mitochondria were noticed in the cytoplasm. Nuclei of the adreno-cortical cells were shrinkage in appearance. On the pineal gland showed large numbers of dark and dead mitochondria within the centre of the pinealocytes. Free ribosomes were less in number. Fluid filled and dark Gap junctions were also noticed. The overall ultra-structural studies of adrenal and pineal gland in male pigeon in various seasons were studied and these results were considered an inverse relationship due to various environmental and experimental condition.

Keywords: Adrenal, melatonin, photoperiod, pigeon, testosterone propionate hormone.

Introduction

The pineal gland acts as a photo neuro-endocrine transducer in birds[1] suggesting that changing environmental conditions may be transmitted by the pineal to act upon adrenal gland and gonadal function during the annual cycle. The involvement of photoperiod in circadian system is a potent environmental stimulus that affects reproduction in birds, seasonally. The increasing day length of late winter and spring serves as the basic environmental information for the control of the annual cycle in the development and function of the gonads of many avian species.[2] Long photoperiods stimulate reproductive development in juveniles and maintain gonadal function in adults. Short photoperiods, on the other hand, inhibit reproductive development and also induce gonadal regression.[3] In birds, light is also known to influence the migratory behaviour, hyperplasia and fat deposition.[4] Exogenous melatonin is capable of increasing bile production by preserving the functional and energetic status during warm ischemia / reperfusion associated with reduced concentration of tumor necrosis factor

(TNF-α) and inhibition of expression of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production.[5] Specific receptors for melatonin have also been identified in avian adrenal glands. Zeman et al.,[6] demonstrated that exogenous administration of melatonin decreased corticosterone level in Japanese quail. It is well established that testosterone regulates avian behaviors such as song and aggression during the breeding season. Testosterone implants increased plasma androgen levels.[7] The exogenous testosterone could act as an active modulator of pineal metabolic activity.[8] Exogenous testosterone caused inhibition of pinealocyte activity in two sexually regressed avian species, Blossom-headed parakeet and the Indian weaver bird.[9] The present sets of experiments have been made to evaluate the effects of exogenous MEL & TP and photoperiodism on the pineal - adrenocortical tissues of the male domestic pigeon, Columba livia Gmelin through ultra-structural examinations.

Materials and Methods

All the birds were collected time to time from the local bird supplier. The pigeons were collected at the first week of the primary breeding phase (i.e., March) and first week of regressive phase- I (i.e., June) every year during the period of 2002-2003, 2003 - 2004 of the investigation. After collection, all the birds were maintained in the uniform laboratory conditions (photoperiod and temperature) for at least seven days. They were provided with food and water ad-libitum. In Experiment-I, at least forty eight birds were used during the period of investigation. For the purpose of study, the male domestic pigeon (Columba livia Gmelin) were exposed to four different conditions of photoperiods (i.e., 12L : 12D, 12L : 12D + MEL., 24L : 0D + MEL and 0L : 24D + MEL) (Melatonin, Sigma chem. Co. Mo., USA, dissolved in a vehicle of ethanol : normal saline) at a daily dose of 20 µg/ 100gm body weight for fifteen days during the primary breeding and regressive phase-I in the evening hours (5: 00 pm).

In Experiment-II, some birds (48 birds) were also maintained under photoperiods of constant illumination and constant darkness (12L : 12D, 12L : 12D + T.P., 24L : OD + T.P., 0L : 24D+ T.P.) in both phases for a period of fifteen days. For this study, the male domestic pigeon were exposed to Testosterone propionate, Sigma chem. Co. Mo., USA, dissolved in a vehicle of ethanol : normal saline at a daily dose of 1 mg/ 100gm body weight for sixty days during the primary breeding and regressive phase-I in the evening hours (5: 00 pm).All the birds were weighed and sacrificed. pineal and adrenal glands were removed promptly for transmission electron microscopic study. For the ultra-structural study of the adrenal and pineal gland, tissues (1mm x 1 mm in size) were fixed in modified Karnovsky's fluid (4% paraformaldehyde and 1% glutaraldehyde), buffered with 0.1 M sodium phosphatase buffer at pH 7.4 (David et al., 1973). Fixation was made for 10-18 hours at 40C temperature, after which tissues were washed in fresh buffer, and post- fixed for two hours in 1% Osmium Tetraoxide in the same buffer at 40C. After several washing in 0.1 M phosphate buffer, these tissues were dehydrated in graded acetone solutions and embedded in CY 212 araldite. Ultra-thin sections of 60-80 nm thicknesses were cut using an ultra-cut E (Reichert Jung), ultra-microtome and the sections were stained in n alcoholic uranyl before examining the grids in a TE M microscope (Philips, CM-10) operated at 60-80k

Results

Experiment-I

During the 12L : 12D photoperiodic condition of the primary breeding phase for 15 days, the ultra-structural studies of the adrenal and the pineal glands of pigeons have been demonstrated. The adrenal glands of birds were characterized by the presence of moderate number of mitochondria filled with vesicles. Light and dark cells were found in the cytoplasm. Lysosomes and lipid granules were quite lesser. Golgi complex and smooth endoplasmic reticulum (SER) were moderate in numbers. Generally, five cell types have been identified in pineal gland. These are the hormone producing pinealocyte, the interstitial cell, the perivascular phagocyte, neurons and peptidergic neuron-like cells. Mitochondria and dense cored vesicles were found to be moderate within the pinealocyte cell cytoplasm. Golgi bodies and lysosomes were moderate in number. Rough endoplasmic reticulums were quite lesser. In 12L: 12D + MEL in primary breeding phase for 15 days, ultra-structural changes within the adrenal and pineal glands were noticed when the pigeons were treated with 12L: 12D photoperiod and exogenous melatonin. Within the adrenal gland, the number of mitochondria was moderate in size. Lysosomes and lipid granules were numerous. Golgi complex and smooth endoplasmic reticulum (SER) were maximal in number. The sizes of the pinealocytes were increased. Mitochondria were found to be minimum within the pinealocyte cell cytoplasm. Golgi bodies and lysosomes were moderate in size. Rough endoplasmic reticulum was few in number. Microtubules were moderate in number. With constant illumination and melatonin treatment for 15 days, the number of mitochondria and lipid granuoles were increased and lysosomes. Golgi complex and smooth endoplasmic reticulum were decreased than the control birds. On the other hand, in both the experiments, the sizes of the pinealocytes were decreased minimally. The Golgi complex showed the typical dictyosomal structure. Mitochondria were oval and long in shape and no remarkable changes were noticed in the number of mitochondria. Numerous free ribosomes were

observed in all the studied areas. Constant darkness with melatonin treatment the adrenocortical cells regions in comparison to the 12L : 12D + MEL birds, the numbers of mitochondria was decreased and they were in hypertrophied condition. The number of lysosomes, lipid granules, Golgi complex and smooth endoplasmic reticulum were increased than the control birds. On the other hand, in both the experiments, the sizes of the pinealocytes were increased minimally. Cytoplasmic portion of the pinealocyte showed pro-centrioles or assembly areas of high electron dense and numerous microtubules, poly-ribosomes and rough endoplasmic reticulum. In 12L: 12D in Regressive phase-I for 15 days, adreno-cortical mitochondria showed typical lamellar or tubular cristae. The smooth ER was essentially more common. But numerous lipid droplets and rounded nuclei were noted in the cytoplasm. Here only light cells were visible. Golgi complex was found nearer to the nucleus also. Pinealocytes were observed as light and dark pinealocytes having greater nucleolar area. Pineal cells of the receptor types were rich in mitochondria, a prominent Golgi apparatus and granular endoplasmic reticulum. The apical surface bears microvilli. Pinealocytes were terminal. Neuropeptide vesicles and clear peptidic vesicles were noted in the cytoplasm. In 12L: 12D + MEL in Regressive phase-I for 15 days, mitochondria were few in number in both the sub-capsular and central zones of the adrenal gland. Lysosomes and lipid granules were numerous. Golgi complex and smooth endoplasmic reticulum (SER) were also few. The pinealocytes were moderate in size and pinealocytes organelles have no any remarkable variations. Constant illumination with induction of melatonin treatment (24L : 0D + MEL) for both the experimental birds in the regressive phase – I exhibited similar ultra structural characteristics in the adrenocortical cells and in the pinealocytes as control (12L: 12D + MEL).

Experiment-II

In 12L: 12D + TP in primary breeding phase for 15 days, within the adrenal gland, the numbers of mitochondria were moderate but these were much enlarged in size. Lysosomes, Golgi complex, smooth ER and lipid granules were minimal in number. Constant illumination and with induction of testosterone propionate (24L : 0D + TP) for 15 days, the numbers of mitochondria were decreased within the adrenal gland. Some dead mitochondria were noticed in the cytoplasm. Nuclei of the adreno-cortical cells were shrinkage in appearance. The membrane and its cristae were very dark and broad. Lipid droplets, ER were increased in number. On the other side, large numbers of dark and dead mitochondria were noticed within the centre of the pinealocytes. Large numbers of neuropeptide containing vesicles were observed near the membrane of the pinealocytes. But Golgi bodies did not show any change. Cilia were noticed in moderate amounts. The sizes of the pinealocytes were decreased maximally. Free ribosomes were less in number. Fluid filled and dark Gap junctions were also noticed. In 0L : 24D + TP in primary breeding phase for 15 days, the mitochondria were moderate in size and number within the adrenal and pineal glands. Lipid droplets and smooth endoplasmic reticulum were also moderate within the adreno-cortical region and pineal gland. Golgi complex did not show any change. Ultra-structures of the adrenal and pineal gland of the pigeons have been demonstrated during 12L : 12D + TP treatment in regressive phase-I. Mitochondria were increased in size and number in both the sub-capsular and central zones of the adrenal gland. Lysosomes and lipid granules were at low level. Golgi complex and smooth endoplasmic reticulum (SER) were moderate. The pinealocytes were decreased in size. Nuclei were very prominent. There was no change in the microtubules. Constant illumination with the administration of testosterone propionate for the experimental birds in the regressive phase – I exhibited, the numbers of mitochondria were increased. But some dead mitochondria were noticed. Lipid droplets, ER were decreased in number. On the other hand, the sizes of the pinealocytes were decreased maximally. Free ribosomes were less in number. Distinct Golgi complex was found within the pinealocytes. The numbers of mitochondria were decreased. In 0L : 24D + TP in Regressive phase-I for 15 days, mitochondria were moderate in size and numbers within the adrenal and pineal gland. Lipids granules,

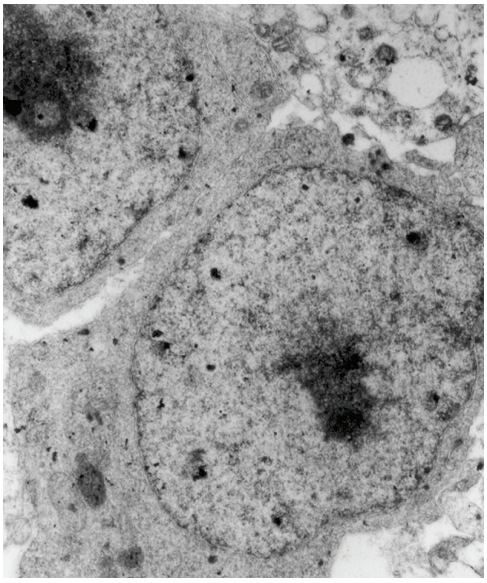

Figure 1: TEM (4200x) of the pinealocyte region after (12L: 12D + MEL treatment) showing increased pinealocyte cell size with distict nucleus and few number of mitochondria during regressive phase-I

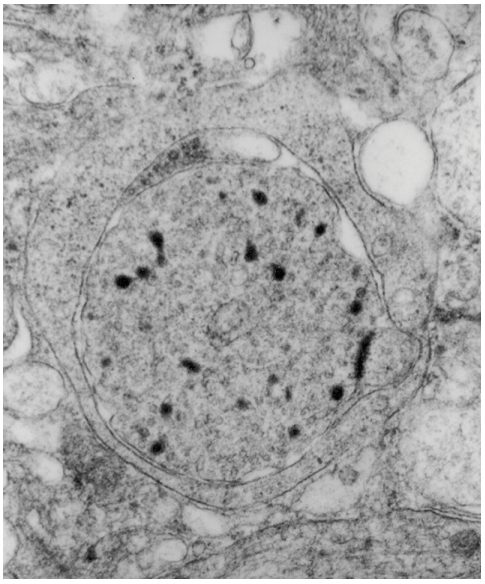

Figure 1: TEM (4200x) of the pinealocyte region after (0L: 24D + MEL treatment) showing increased large number of vesicles during regressive phase-I

Golgi complex and smooth endoplasmic reticulum were also few within the adreno-cortical regions and pineal gland. Pinealocytes were moderate in size.

Discussion

Research on circadian rhythms has focused on the photoperiodic cues involved in the regulation of avian reproduction. The significance of exogenous melatonin administration is suggestive of an antigonadal effect. In the present study, the body and the pineal gland weight have been decreased due to the exogenous melatonin treatment which exerted a negative feedback effect on the biosynthesis and secretion of melatonin by the pineal gland but the pineal gland was also active. The exogenous melatonin induced hyperactivity of the adrenal cortex and regressed gonadal function during non-breeding phases. But, the adrenal gland showed atrophic condition and regressed the gonadal function during the breeding phase. In the non-breeding phase, with continuous darkness and evening melatonin treatment schedule increased adrenal gland and decreased pineal gland weight than the control birds. But melatonin activity coincided with the maximum adrenal function in the long photoperiods during non-breeding phase and melatonin treatment evoked signs of significant hyperactivity of the pineal gland during the breeding phase. The present ultra-structural study of the pineal gland of pigeons after exposure to continuous darkness with induction of melatonin during breeding and non-breeding phases, revealed enlarged mitochondria, numerous polyribosomes, rough endoplasmic reticulum and microtubules. Microtubules are involved in secretory processes. In the present work melatonin treatment augmented pineal microtubule protein levels and increased the number of microtubules within pineal cells of the pigeons. Enlarged size of the mitochondria under constant darkness conditions may also be considered important in view of the pineal gland. But after constant light with melatonin treatment, the number of mitochondria were increased and lipid droplets, Golgi complex, SER were decreased in the adrenocortical regions than the control birds with melatonin groups (12L : 12D), suggests the need for the high energy requirements related to the process of cellular function. Mitochondria are considered to be the main source of free radicals in the cell and oxidants produced by the electron transport chain. But treatment with testosterone propionate, the results indicated that testosterone inhibited pinealocyte function in the adult male domestic pigeons in both the breeding and non-breeding phases. These results indicated that the sex steroid acted on the pinealocytes through systematic circulation and are active modulators of pineal metabolic activity and change the rate of synthesis of pineal hormones in birds. It is suggested that an exogenous TP reduces the normal synthetic-secretory activity of the pineal gland. It can be assumed that with the administration of exogenous TP, pineal gland did not discharge its secretory product and no synthetic mechanism occurred. The adrenal and the pineal gland, broad and continuous types of cristae within the mitochondria are found and many mitochondria are degenerated, suggested that due to less endogenous melatonin content it lead to increase the harmful induction in the mitochondrial membrane potential that have failed to trigger the mitochondrial transition pore opening and ultimately resulted in the apoptotic cascade. Melatonin mitigates also mitochondrial malfunction (Leon et al., 2005). Exogenous testosterone propionate exerted inhibitory influences on the testicular, adrenal and pineal functions when it was administered to primary breeding phase. But the results were stimulatory on testicular-adrenal function and inhibitory on the pineal function when the birds were in the regressive phase-I. Exogenous TP induced atrophy of the adrenal cortex during both the phases. It might be more accurate to think that MEL & TP acts as the major endocrine input to a calendar system that serves as the reference for the timing of the reproductive rhythms and its biological rhythms.

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