Influence of photoperiods on glycemic and adrenal catecholaminergic responses to melatonin administrations in adult male roseringed parakeets, *Psittacula krameri* Neumann

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Effects of daily (one hour prior to onset of darkness) injection of melatonin (25 μg/100 g body wt. for 30 days) on concentrations of blood glucose and adrenal catecholamines were studied in adult male roseringed parakeets, *P. krameri* under both natural (NP; about 12L:12D) and artificial long (LP; 16L:8D; lights were available in between 0600 and 2200 hrs) or short (SP; 8L:16D; lights were available between 0600 and 1400 hrs) photoperiodic conditions. The results indicate that neither LP nor SP as such exerts any significant effect on blood glucose titre of control (vehicle of hormone administered) birds. Treatment with melatonin, however, induced hyperglycemia in both NP and LP bird groups, but hypoglycemia in SP birds. Unlike glycemic levels, amount of epinephrine (E) and norepinephrine (NE) in adrenals of control birds exhibited significant changes under altered photoperiods. A decrease in E and an increase in NE were noted in adrenals of both LP and SP birds. Exogenous melatonin in NP birds also caused a decrease in E and concomitant rise in NE levels. On the other hand, treatment of melatonin in both LP and SP bird groups resulted in an increase in the quantity of both E and NE compared to respective values in adrenals of melatonin injected NP birds. However, relative to the amount of E and NE in adrenals of placebo treated LP and SP birds, significant effect of melatonin treatment was observed only in SP birds. The results suggest that influences of exogenous melatonin on the levels of both blood glucose and adrenal catecholamines are largely modulated by short rather than long photoperiods.

The pineal gland acts as a neuroendocrine transducer which exhibits well-defined diurnal melatonin output as a response to photic stimuli and thus influences a number of metabolic and endocrine functions. The role of pineal gland and its hormone melatonin in the regulation of carbohydrate metabolism has been studied in mammals, as well as in a few species of birds, including pigeons and roseringed parakeets. Attempts have also been made to demonstrate the influence of the pineal gland and/or its hormone melatonin on the functions of avian adrenal medulla. However, any functional relationship between the levels of adrenal catecholamines and blood glucose in the melatonin treated birds is as yet a topic of speculation. Likewise, despite recognition of the pineal organ as an intermediary between the environment and endocrine system in birds, it was largely unexplored whether effects of melatonin on the levels of blood glucose and adrenal catecholamines are modulated by the changes in photoperiod. Accordingly, it was envisaged in the present investigation to demonstrate the glycemic and adrenal catecholaminergic responses to exogenous melatonin in adult male roseringed parakeets under natural and altered photoperiods.

Materials and Methods

*Collection and maintenance of birds* — Adult male roseringed parakeets (*Psittacula krameri* Neumann; *Psittaciformes; Aves*) were collected locally (Lat. 23°14'N; Long. 87°51'E), during February (natural photoperiod: around 11 hr 30 min) corresponding to the breeding phase in an annual testicular cycle. The birds were acclimatized for about a week in an open-air aviary under natural photoperiods, and 20-45% RH, and were provided with paddy (*Oryza*) as food and water *ad libitum* during acclimatization and throughout the experiment.

*Experimental paradigm* — A total of 36 birds weighing between 110-140 g were divided at random into three groups. Each group, containing 12 birds, was separately held under natural (NP), or long (LP; 16L:8D; artificial lights were available in between 0600 and 2200 hrs), or short (SP; 8L:16D; artificial lights were available in between 0600 and 1400 hrs) photoperiods. NP birds were maintained in an open-air aviary under natural conditions including the photoperiods which varied between 11 hr 30 min (at the onset of experiment) and 12 hr (at the end of experiment), though supplementary artificial lights...
were available in the shaded areas of the aviary between 0800 and 1700 hr. In all cases artificial lights were provided with white fluorescent lamps (intensity of light varied between 250-300 lux at cage level). The birds of either photoperiodic group were subsequently divided into two sub-groups each containing 6 birds. The first sub-group received only the placebo solution (saline-ethanol mixture in 9:1 v/v ratio) and were considered as the control for the melatonin treatment. The birds belonging to the other sub-group, under each photoperiodic schedule, received i.m. injection of melatonin (Sigma Chemicals, USA) at the dose of 25 μg/100 g body wt/ day for 30 consecutive days. This dose schedule of melatonin has been used by many investigators to study the ‘melatonin effect’ in mammals and is still used in the study on birds. Though the influence of melatonin on avian gonad has been reported to be independent of the time of administration, generally exogenous administration of melatonin is done just before the onset of darkness in a 24 hr cycle, as this schedule has shown maximum efficacy of the treatments in several mammalian studies. Accordingly, the parakeets to each photoperiodic group were injected daily with melatonin or its vehicle at the end of respective light phase.

Collection of blood samples and adrenals — On the following day of the last treatment, the blood sample (200 μl) from each bird was collected separately from the brachial vein during mid-noon, i.e. at around 12.00 noon when blood glucose level reaches its peak in parakeets. Subsequently, the birds were sacrificed by cervical dislocation, and quick dissection was followed for the collection of both the adrenal glands from each individual. After removal of extraneous tissues, the paired adrenals were soaked with blotting paper, weighed in a balance sensitive to 0.01 mg, and transferred to pre-chilled tubes containing 6% TCA solution.

Measurement of blood glucose levels — Quantity of glucose in blood was estimated by glucose oxidase-peroxidase method using ortho-dianisidine as the colour reagent. The value was expressed as mg of glucose/dl of blood.

Measurement of concentrations of adrenal catecholamines — The adrenals were homogenized in 6% TCA, purified for quantitative determination of the amounts of epinephrine (E) and norepinephrine (NE) according to a method described elsewhere. The concentrations of E and NE in the adrenals were expressed as μg/mg of tissue.

Analysis of data — The data were analysed by two-way analysis of variance (ANOVA) followed by two-tailed Student's t test. The criterion for significance was P ≤ 0.05.

Results

A. Influences of altered photoperiods on the concentrations of blood glucose and adrenal catecholamines in control birds — The mean values of the concentrations of blood glucose, adrenal E and adrenal NE in different bird groups have been presented in Figs. 1 and 2 respectively. The blood glucose level in control (vehicle of melatonin injected) birds did not vary significantly with regard to the changes in photoperiods (Fig. 1). However, altered photoperiods (both LP and SP) induced a significant decrease in the concentration of E (Fig. 2A) and a concomitant rise in the level of NE (Fig. 2B) compared to the values in the adrenals of NP birds.

B. Effects of melatonin on the concentrations of blood glucose:

(i) Under natural photoperiodic conditions — Daily administrations of melatonin to NP birds resulted in hyperglycemia (Fig. 1) compared to the blood glucose values in control birds.

(ii) Under altered photoperiodic conditions — Glycemic response in parakeets to exogenous melatonin

![Fig. 1 — Diagrammatic presentation of the values (means±S.E.'s in vertical bars) of blood glucose level in natural (NP; about 12L:12D), long (LP; 16L:8D), and short (SP; 8L:16D) photoperiodic male roseringed parakeets following daily administration of vehicle of hormone (control) or melatonin for 30 consecutive days. [Significant at 5% level, compared to control group under identical photoperiodic condition; Significant at 5% level, compared to corresponding group under natural photoperiodic condition.]
C. Effects of melatonin on the concentrations of adrenal catecholamines:

(i) Under natural photoperiodic conditions—
Exogenous melatonin induced a significant reduction in the concentration of E (Fig. 2A) and a concomitant increase in the level of NE (Fig. 2B) in the adrenals of birds maintained under natural photoperiodic conditions.

(ii) Under altered photoperiodic conditions—
Compared to the values in plebeo (control) group of LP birds, no significant change was observed in the levels of adrenal E (Fig. 2A) and NE (Fig. 2B) in the melatonin treated LP birds. However, the concentrations of both E and NE were found to be significantly higher in the adrenals of LP birds than those measured with the adrenals of melatonin treated NP birds. On the other hand, a significant increase in the concentrations of both E (Fig. 2A) and NE (Fig. 2B) was noted in the adrenals of melatonin administered SP birds compared to the concentrations of respective adrenal catecholamines in control NP as well as in control SP groups of parakeets.

Discussion

In mammals, light information is transferred from the eyes to the pineal where it controls the production of melatonin. This idea has an implication that the lighting environment may play a role in the regulation of endogenous level of melatonin. Although this pathway is not yet clear in avian species, Liou and Liou et al. implicated both the light-dark cycle and melatonin in the entrainment of oviposition suggesting that the role of light and melatonin in birds is similar to that in mammals.

The results of the present study revealed that under natural photoperiodic conditions, the treatment of melatonin in parakeets results in hyperglycemia. Previous studies on pigeons following a single intravenous injection or subcutaneous implantation for a 12-week period of melatonin also demonstrated similar effect on the level of blood glucose. However, reports are available to show that glycemic response to melatonin administration may vary in relation to the time period during a circadian cycle or an annual reproductive cycle at which melatonin is injected, and also the dose of administered melatonin.

In consideration to the data in the literature and the observation made in this study, it is not surprising that the treatment of melatonin in male roseringed parakeets during the inactive phase in an annual gonadal cycle resulted in hypoglycemia, and an identical treatment in the sexually active birds of the same species caused hyperglycemia (present study). The effects of pinealectomy on the blood glucose level...
and on the metabolism of carbohydrate were also shown to vary in relation to the reproductive season in an annual cycle. These observations were taken to indicate a possible anti-insulinic/anti-avian pancreatic polypeptide role for the pineal during the breeding phase and reverse actions of the pineal principle during the non-breeding phase in an annual gonadal cycle in wild pigeons. However, at present it is difficult to confirm a synergistic or antagonistic interaction between the pineal and pancreas in relation to the breeding activity of roseringed parakeets.

The role of photoperiods on the regulation of gonadal functions has been well studied in several avian species, including the roseringed parakeets. Under natural photoperiodic conditions recovery of gametogenesis from the prolonged resting phase in an annual testicular cycle in parakeets occurs after experiencing autumal equinox, but active gametogenesis in the testis takes place only after winter solstice (corresponding to photoperiods of about 10 hrs 30 min) under the influence of increasing photoperiods ranging between 11 and 12 hr.16. A positive correlation has also been noted between the rate of testicular growth in free-living parakeets and the duration of environmental photoperiods in annual cycle.16 However, the authors are not aware of any previous study on the influence of altered photoperiods on the glycemic level in any bird. It is evident from the results of the present study that neither LP nor SP has any significant effect on the concentration of blood glucose in control (vehicle of melatonin injected) parakeets. The glycemic response to melatonin was also identical among the NP and LP birds. But the level of blood glucose in melatonin administered SP birds was found to be significantly reduced compared to the glycemic values in both the control SP and melatonin injected NP parakeets. The results suggest that the duration of light as such may not have any influence on the blood sugar level in parakeets, but glycemic effect of melatonin is essentially dependent on the photoperiodic conditions under which the birds are held. In all probability, a short photoperiodic schedule may act as a modulator of the physiological responsiveness mechanism to the regulation of blood glucose level in the melatonin challenged bird. It may be noted here that exposure of hamsters to SP was also found to modify the effects of melatonin on the gonad.34.

Administration of melatonin in NP parakeets resulted in a significant decrease in the amount of epinephrine (E) and a concomitant increase in the level of norepinephrine (NE) in the adrenals. Earlier studies on different mammalian species have shown that the pineal organ play an important role in the regulation of adrenal catecholaminergic functions. Administration of melatonin may cause an increase as well as a decrease in secretory activity depending on the cell types of adrenal medulla.35 A study on the adrenal medulla of different species of birds following 0.5 hr of a single intraperitoneal injection of melatonin (0.5 mg/100 g body wt.) revealed a species specific and age specific response of E and NE cell.33. The quantitative estimations of catecholamines in the adrenals of pinealectomized and/or melatonin treated young chicks also demonstrated differential behaviour of E and NE in the adrenals of concerned birds.34. The biosynthesis of catecholamines in an avian adrenal medulla proceeds, probably following the mammalian pattern, from tyrosine to dopa to dopamine to norepinephrine to epinephrine.36. The conversion of NE to E requires methylation of NE by the enzyme phenylethanolamine-N-methyl transferase or PNMT.37. In light of the existing knowledge, it may not be unwise to speculate that the increase in the amount of NE accompanied by the stoichiometric decrease of E in the adrenals of melatonin treated birds may result from: (a) an increased synthesis of NE by melatonin probably through inducing ACTH release, that in turn activated tyrosine hydroxylase directly and/or (b) a direct or indirect inhibition of the activity of PNMT.

Unlike the levels of blood glucose, the concentrations of adrenal E and NE in different groups of control bird were found to undergo significant changes in relation to the schedule of photoperiod. Exposure of birds to both LP and SP schedules evoke an identical response in the level of adrenal catecholamines. In either case, a significant decrease in the concentration of E was accompanied by a concomitant increase in the level of NE compared to the respective values in the adrenals of NP birds. Although the functional etiology of the photoperiodic response of adrenal catecholamines remains unknown, the study on rats under continuous illumination and darkness also revealed identical pattern of synthesis and metabolism of adrenomedullary hormones.37. It has been suggested that one-month continuous exposure of rats to either light or darkness may lead to an appreciable decrease in the amount of E and the E synthesizing enzyme (PNMT) in the adrenals. There is a possibility that an altered
photoperiodic schedule modifies the tone of the sympathetic nervous system\(^1\)\(^,\)\(^2\) related to the secretion of catecholamines by the adrenal gland.

The data on the adrenal catecholamines in the melatonin administered altered photoperiodic birds are interesting. The concentrations of adrenal E and NE in melatonin treated LP birds were not significantly different from those in the control birds held under LP schedule. On the other hand, the values of both E and NE in the adrenals of melatonin injected SP birds were found to be significantly increased over the concentrations of respective adrenal hormones in control SP as well as melatonin treated NP birds. This variability between the influences of LP and SP on the glycemic and catecholaminergic effects of melatonin in parakeets may be explained as due to difference in the duration of melatonin to act to elicit its physiological response\(^3\). In fowl prolonged darkness is known to modulate the endogenous milieu of melatonin\(^4\).

Collectively, the results of this study indicates that the light/dark ratio influences either directly or indirectly the endocrine system\(^5\) involved in the regulation of adrenal catecholaminergic as well as glycemic responses to exogenous melatonin. In this respect, probably SP schedule is more effective modulator of melatonin effect than the LP schedule. The conjecture earns support from the results of earlier workers who have demonstrated that the synthesis of melatonin becomes significantly higher in the pineal of hamsters housed under short photoperiods\(^6\) and melatonin effects on the reproductive system is modified to larger extent by SP than LP in seasonally breeding mammals\(^7\).

Nonetheless, the topic should remain speculative until measurement of melatonin titre in the currently considered avian species under given experimental conditions.

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References

8. Ramachandra A V & Patel M M, Seasonal alterations in carbohydrate metabolisms revealed by tissue glycogen contents and blood glucose levels of normal and pinealectomised domestic pigeons, Columba livia (Gmelin), Montiure Zoöl Iatel (NS), 21 (1987) 11.


22 Stetson M H & Tay D E, Time course of sensitivity of golden hamsters to melatonin injections throughout the day, Biol Reprod, 29 (1983) 432.


41 Januszewicz W & Wocial B, Influence of work, the daily rhythm and sight on the excitation of catecholamines in the urine, Arch Med Wiss, 30 (1960) 207.


46 Reiter R J, Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals, J Neural Transm(Suppl), 21 (1986) 35.