

EFFECT OF SINGLE ORAL DOSE OF MELATONIN ON PLASMA CONCENTRATION OF PROLACTIN AND CORTISOL IN MALE RHESUS MONKEYS

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Background: Melatonin is a neurotransmitter involved in physiological regulation; it is thought that melatonin has a role in male reproductive physiology through effect on neuroendocrine axis. The present study was aimed to investigate the effect of melatonin on plasma levels of Prolactin and Cortisol (ng/ml). **Methods:** Five adult male rhesus monkeys were used for the purpose. Monkeys were maintained at *ad libitum*. Melatonin 5 mg was administered orally to all five monkeys. Sequential blood samples were collected at 15 minute intervals in heparinised syringes. Plasma Prolactin and Cortisol were analysed by ELISA and RIA respectively. **Results:** Melatonin oral single dose (5 mg) increased the plasma prolactin concentration in all five monkeys after 15 minute of treatment and this increase remained until 60 min but this increase was not significant compared to before treatment concentration. The highest mean concentration of prolactin was observed at 15 to 60 minute (6.38±0.18 ng/ml) and the lowest mean concentration was observed between 75 to 120 minute interval (5.43±0.22 ng/ml). Decrease in the later was significant compared to former ($p<0.001$). Present study results showed significant effect of melatonin in terms of reduction on plasma prolactin concentration ($F_{(2,52)}=3.989$; $p<0.05$). The study results showed no significant effect of melatonin on plasma Cortisol concentration ($F_{(2,52)}=0.074$; $p>0.05$) in adult male rhesus monkeys. **Conclusion:** Our study suggests positive role of melatonin in reducing pathological effects of Prolactin, while it has been revealed that stress has no physiological coupling with melatonin administration.

Keywords: Melatonin administration, Plasma Prolactin, Plasma Cortisol,

INTRODUCTION

The pineal gland is an important mediator of the photoperiodic time cues through which seasonal breeders respond to the annual cycle in day length.¹ The pineal secretes melatonin, the blood concentrations of which increase during the hours of darkness. The hormone secretion is stimulated by dark and inhibited by the day.²

There are clear evidences about melatonin to modify the immunity, stress responses, reproductive physiology and aging and is thought to be key factor in regulation of the seasonal variation in gonadal activity by its actions at various levels of hypothalamic-pituitary-gonadal axis.² A 'hormone of darkness' has been shown to affect the plasma concentration of various hormones like prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and growth hormone (GH).³ Melatonin modulates the neuroendocrine axis in male infertility, because low levels of melatonin were present in the semen of infertile patients in one authentic study.

Prolactin, a 23 KD hormone, synthesised in the adenohypophyseal lactotrophs, has no known target organ or defined role in male reproduction, although functional significance of the prolactin in reproduction is unequivocally explained but still prolactin is involved in male infertility.⁴ Research

results reported during the past 25 years have added to the evidences that pathological hyper secretion of prolactin (hyperprolactinemia) in men is associated with hypogonadism and/or impotence and that the effects of prolactin on the central nervous system control of gonadotropin release and sexual behaviour are important in the aetiology of these disorders.⁴

Prolactin promotes the hyper secretion of the adrenal corticoids and inhibits the secretion of gonadotropin releasing hormone (GnRH) through receptors on dopaminergic neurons which leads towards hyperprolactinemia, one of the major cause of male infertility. Over production of prolactin also leads toward the productions of lactotrophs adenomas both in males and in females. Melatonin suitable doses are shown to inhibit the oestrogens induced prolactinomas development by inhibiting the prolactin gene expression and DNA synthesis.⁵ However melatonin significantly reduced the inhibitory effects of dopamine on prolactin secretion.⁶ There is evidence of melatonin induced inhibition in prolactin concentration at suitable doses in silver fox mating season.⁷ But in contrast to the report above, the melatonin long term therapy is thought to reduce the sperm quality in healthy men.⁸

Cortisol is a glucocorticoid hormone that is involved in the response to stress; daily variation of glucocorticoid levels in blood is a classical example

of circadian rhythms. It has been demonstrated in several mammalian species including humans^{9,10}, rats^{11,12}, pigs^{13,14}, rhesus monkey¹⁵. In several vertebrates, stress has been shown to interfere with reproduction and the functioning of the brain pituitary-gonadal (BPG) axis.¹⁶ Charpenet *et al.* demonstrated that chronic intermittent immobilization stress induced a strong decrease of plasma testosterone levels and testicular testosterone content in rats.¹⁷

Variety of animal studies has shown relationship of pineal gland with hypothalamic-pituitary-adrenal axis. Melatonin might be involved in stress producing response because in one study a suitable dose of melatonin is thought to advance the peak of Cortisol and increase the Prolactin levels.¹⁸

In one published report on healthy male subjects cortisol secretion was unaffected by sleep deprivation, regardless of melatonin presence or absence.¹⁹ In young adults melatonin onset typically occurs during Cortisol quiescent period and with a time lag the start of quiescent period and melatonin onset of 90 min.²⁰ Some reports have shown to reduce the circulating Cortisol concentration after melatonin administration.²¹

These reports and data showed controversial role of melatonin in modulating the plasma levels of hormones of clinical implication like prolactin and Cortisol. In order to investigate the effect of melatonin on these important hormones the study has been designed to investigate the effect of exogenous melatonin on the secretion of plasma Prolactin and Cortisol concentration ($\eta\text{g/ml}$) and to determine whether melatonin could act directly on pituitary to modulate PRL and Cortisol secretion in adult male rhesus monkeys (*Maccaca mulatta*).

MATERIAL AND METHODS

Five adult male rhesus monkeys (*Maccaca mulatta*), 7.5 to 8 years of age, were used in this study. The animals were given numbers as 1, 2, 3, 4 and 5. The body weight of the animal at the time of the experiment ranged from 8.6–10.4 Kg. The animals were housed in individual cages, were maintained under standard colony conditions at the Primate Facility of the Quaid-i-Azam University, Islamabad. They were provided with standard monkey food supplemented with fresh fruits and vegetables. Water was available *ad libitum*. The animals were habituated for chair restrains 8 weeks prior to the commencing of the experiment.

Before handling, the animals were anaesthetised with ketamine (5 mg/kg; Ketavet, Parke-Davis, Freiburg, FRG) and while under sedation, a Teflon cannula (Vasocan Brannule 0.8 mm/22 G.O.D., B. Braun, Melsangen AG, Belgium)

was inserted in the cephalic vein 60 min before initiation of sampling and the animals were restrained to the chair.

Melatonin (N-acetyl-5-methoxytryptamine) (5 mg, General nutrition corporation, Pittsburgh, PA 15222) was administered orally to all five monkeys. Sequential blood samples (2.0 ml each after every 15 min) were obtained at -30, -15 and 0 min before melatonin administration (single dose 5 mg was given orally). After melatonin administration the blood samples were taken at 15, 30, 45, 60, 75, 90, 105, and at 120 intervals. The blood samples were taken in heparinised syringes.

Plasma prolactin concentration in adult male rhesus monkeys was determined by Enzyme-linked Immunosorbent Assay (ELISA). The prolactin enzyme immunoassay kits were obtained from Biocheck, Inc (323 Vintage Park, Dr. Foster City, CA 94404).

Plasma Cortisol concentration was assayed by RIA. Measurement range of the assay was 10-2000 ηM . The Cortisol Radioimmunoassay kits were obtained from Biocheck, Inc (323 Vintage Park, Dr. Foster City, CA 94404).

For evaluation of effect of melatonin administration on prolactin and cortisol concentration the time period was divided into three consecutive segments in order to interpret the results clearly. For data analysis mean and standard error were calculated. Unpaired Student's *t*-test was applied for comparison. One-way Analysis of Variance (ANOVA) was applied to see how plasma Prolactin and Cortisol concentrations differ after treatment with melatonin or with time and to check the significance of response elicited by melatonin dose administration.

RESULTS

Individual plasma Prolactin concentration ($\eta\text{g/ml}$) before and after melatonin (single dose 5 mg) administration is shown in Table-1 and 2.

Table-1: Plasma Prolactin ($\eta\text{g/ml}$) in adult male rhesus monkey before and after melatonin (single dose 5 mg) administration (Mean \pm SEM)

Time (min)	Plasma Prolactin concentration ($\eta\text{g/ml}$) (Mean \pm SEM)
-30	5.55 \pm 1.05
-15	5.42 \pm 0.65
0	5.77 \pm 0.41
15	6.88 \pm 0.40
30	6.44 \pm 0.21
45	6.18 \pm 0.21
60	6.04 \pm 0.48
75	5.69 \pm 0.33
90	5.61 \pm 0.39
105	5.36 \pm 0.59
120	5.08 \pm 0.49

Table-2: Plasma Prolactin concentration (ng/ml) at various segments of before and after melatonin (single dose 5 mg) treatment in rhesus monkeys (Mean±SEM)

	Time (min)		
	Before Treatment		After Treatment
	-30 to 0	15 to 75	90 to 120
Mean±SEM	5.58±0.41	6.38±0.18	5.43±0.22*

* ($p < 0.001$) 15 to 60 Vs 75 to 120 min segment comparison

Mean±SEM Plasma Cortisol concentration (ng/ml) before and after a single dose of melatonin (5 mg) administration is given in Table-3 and Table-4.

Table-3: Plasma Cortisol concentration (ng/ml) in adult male rhesus monkey before and after melatonin (single dose 5 mg) administration (Mean±SEM)

Time (min)	Plasma Prolactin concentration (ng/ml) Mean±SEM
-30	399.94±69.54
-15	331.64±50.96
0	448.15±94.67
15	467.26±83.65
30	387.38±99.24
45	435.67±87.65
60	356.25±74.81
75	324.06±53.26
90	293.83±51.36
105	402.24±77.54
120	428.83±51.16

Table-4: Plasma Cortisol concentration (ng/ml) at various segments before and after melatonin (single dose 5 mg) treatment in adult male rhesus monkey

	Time (min)		
	Before Treatment		After Treatment
	-30 to 0	15 to 75	90 to 120
Mean±SEM	393.9±41.6	393.9±41.46	371.4±92.1

($p > 0.05$) Pre-treatment segment VS 90 to 120 minute segments

DISCUSSION

Melatonin is thought to entrain the organism to light dark changes of environment and thought to have effects in regulation of reproduction like neuroendocrine axis control.² In one study Prolactin has been stimulated and phase advanced after melatonin administration.²² While Terzolo *et al.* in one of his experiment found Prolactin to be unchanged by melatonin action in men.²³ The results of the most of the studies done on seasonal breeders, including the mare showed that, administration of melatonin results in an inhibition of Prolactin secretion in males. The Prolactin axis of the males is inhibited by melatonin only during a specific time of year, namely the spring and early summer months.²⁴ In this study compared to pre-treatment Prolactin concentration, there was an increase in Prolactin concentration after treatment with melatonin, but this increase was not significantly different from that of pre-treatment concentration. The increase in Prolactin concentration was persistent up to

60 minutes after treatment, but afterwards there was decline in Prolactin concentration until the end of the experiment at 120 minute (after minute), this decrease in Prolactin concentration was not significant compared to increased concentration. George *et al.* working on male rhesus monkeys observed no effect of melatonin on basal or TRH stimulated values of Prolactin in male rhesus monkeys.²⁴

Villauna *et al.*²⁵ reported in their work that melatonin influences Prolactin secretion through unknown mechanisms. They studied the effects of melatonin administration on Prolactin secretion in pituitary-grafted male rats. Melatonin administration 5 hours before dark resulted in a marked decrease of previously high basal plasma Prolactin levels in pituitary-grafted rats, whereas a marked increase was detected in sham-operated animals. Villauna *et al.*²⁵ have suggested that, melatonin appears to modify PRL secretion through multiple complex mechanisms that may depend on the physiological status (hormonal and neurotransmitter) of the animals. A direct effect of melatonin at the pituitary level altering lactotroph sensitivity seems to be one reason for their findings, although they thought that a hypothalamic site of action could be a reason also. Similar results have also been reported by Lincoln *et al.*²⁶, who employed hypothalamic-pituitary disconnected (HPD) and control Soay rams treated chronically for 48 week with continuous-release implants of melatonin while under long days (16L: 8D), the implants produced continuously elevated blood concentrations of melatonin 2–3 times higher than the normal nocturnal maximum. The long-term treatment induced a biphasic effect on Prolactin secretion in both the HPD and control rams, with a marked decrease in the blood Prolactin concentrations for 10 week followed by a gradual increase. The introduction of a second melatonin implant after 20 weeks failed to affect Prolactin secretion. Melatonin acts primarily in the pituitary gland to affect Prolactin secretion, and partial refractoriness develops at this level for control of Prolactin.²⁶ Their results are in agreement with our results where melatonin administration resulted in elevation and then suppression of Prolactin secretion.

Present study also showed significant decrease in Prolactin levels in last leg of experiment (75–120min) compared to previous increase (15–60 min) ($F_{(2, 52)} = 3.989$; $p < 0.05$).

Present study supports that melatonin slightly increases the Prolactin concentration by suppressing the dopaminergic pathway because melatonin receptors are present in high affinity in the pars tuberalis which drives the seasonal rhythmicity of the hormone but after an hour the secretion was suppressed may be because melatonin acted primarily on the pituitary gland to affect Prolactin secretion, and

partial refractoriness develops at this level for control of Prolactin which caused a marked decrease in its concentration and this shows time dependent effect of melatonin on plasma Prolactin secretion. Melatonin receptors have been found in SCN of hypothalamus in brain of humans and rodents. Recently nuclear receptors have been found for melatonin including hypothalamus and a functional analogue for melatonin has been isolated that binds to the nuclear receptor but does not bind to the high affinity membrane receptor of melatonin. So might be melatonin act through this nuclear receptor to affect the adeno-hypophyseal system to modulate the Prolactin secretion.

In our study oral melatonin administration caused no significant increase in the Cortisol concentration after 15 minutes of treatment. Melatonin treatment did not modify the Cortisol concentration which was 393.9 ± 41.6 ng/ml before treatment. Cortisol concentration remains unaffected throughout the study period. However, it has been investigated by Forsling *et al*, who conducted study on healthy male volunteers in which melatonin influence on Cortisol secretion was studied. Eight men with mean age of 21 ± 0.5 years were studied, observations being made after the administration of melatonin in doses of 0.05, 0.5 or 5.0 mg or placebo. They refrained from taking heavy exercise, alcohol and from smoking for 24 h prior to the study. Serum cortisol was measured in samples taken at 30 minutes intervals for 150 minutes after the administration of melatonin. There was no significant effect of melatonin on cortisol release after melatonin administration.²⁷ Their results are in total agreement with our results in which melatonin cause no effect on cortisol secretion.

In another study Stefan *et al*, has shown different results with humans. It was concluded from their data that in totally blind individuals the single administration of a clearly pharmacological dose of melatonin (5 mg) can improve sleep function by synchronising in time the inhibition of pituitary-adrenal activity with central nervous sleep processes. Melatonin affects plasma cortisol concentration but it seems that its effects are due to some particular physiological state like blindness in individuals; in normal subjects these results are not reported.²⁸ So our data confirms the previous reports findings that melatonin the pineal hormone has no effect on cortisol concentration and there lies no physiological coupling of these two hormones in mammals because melatonin is the hormone of darkness its peak has been observed during night and cortisol the hormone associated with stress and wakefulness has its peak observed during active period of day in healthy subjects.

CONCLUSION

In conclusion our data is in agreement with some authentic reports that melatonin decrease the Prolactin concentration via some unknown pathways and this finding could be helpful because melatonin pharmacologic doses can be used in treating pituitary adenomas that are produced due to high production of Prolactin and also beneficial in treatment of hyperprolactinemia in male to treat infertility. While in our report stress has found no relationship with melatonin administration.

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