EFFECT OF MELATONIN ON GLUCOSE TOLERANCE AND BLOOD GLUCOSE CIRCADIAN RHYTHM IN RABBITS

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Summary : Experimental studies on pinealectomized animals and *in vitro* studies using pancreatic tissue, have indicated that the pineal gland has a suppressive effect on the pancreatic *B* cells which secrete insulin. In this study, melatonin, was injected into rabbits and a statistically significant decrease in glucose tolerance was noted.

The effect of melatonin in influencing the circadian rhythm of blood glucose was also studied in rabbits. Results showed that melatonin influences the circadian rhythm leading to a shift in the occurence of minimum levels from 16.00 hr to 04.00 hr (next day) during fasting and from 16.00 hr to 20.00 hr during feeding. Also melatonin treatment lead to a statistically significant rise in blood glucose levels.

It is probable that melatonin administration reduces glucose tolerance and influences the blood glucose circadian rhythm mainly through its effects on insulin release by pancreatic *B* cells.

Key words : melatonin, glucose loading, tolerance, circadian rhythm, blood glucose

INTRODUCTION

The pineal gland has been shown to influence the activity of endocrine glands of ectodermal origin (6). By noting the effects of pinealectomy in rats, (which resulted in increased activity of *B* cells of Langerhans' islets and hence hypoglycaemia) it was postulated (7) that the pineal gland suppresses the activity of the *B* cells. On the other hand, the effects of melatonin, (the principal hormone of the pineal gland) vis-a-vis serotonin and other indol-amines, on glucose stimulated insulin release, was compared (9). It was found that melatonin had no effect whereas serotonin was the most potent inhibitor in this series. It is possible that the original results, based on the effects of pinealectomy (7) may have been so, as a result of removing the inhibitory effect of serotonin (present in the pineal gland) on insulin release from the *B* cells. Serotonin is the precursor of melatonin. Again it was reported that melatonin administered in cynomagolous monkeys resulted in increased blood glucose levels, indicating suppression of insulin release (3).

In view of these conflicting reports, the present trials were undertaken to ascertain the effects of melatonin on insulin release, by studying tolerance to glucose loading in rabbits. Also, since a circadian rhythm of blood glucose exists (8, 10 11, 15, 16) and the pineal gland controls circadian rhythm, the effect of melatonin as a possible controller of this diurnal rhythm was studied.

MATERIAL AND METHODS

In both sets of experiments, 20 adult healthy rabbits of either sex, weighing around 1.5 *kg* were used. Melatonin (Sigma) dissolved in methanol-normal saline was injected intramuscularly in a dose of 5 *mg/kg* in the experimental animals, whereas, the control animals received only the solvent in equal amounts. Blood samples were drawn from the marginal ear vein, into fluoride vials, and the blood glucose estimated by Nelson-Somogyi method.

Influence on glucose tolerance in rabbits : Ten animals served as controls and the rest as experimental ones. Animals of both these groups were fasted for 16 hr prior to the start of the experiment, to get uniform fasting conditions. Water was given *ad libitum*. At the onset of the experiment, glucose was passed orally via the Ryle's tube 1 *g/kg* in all animals. After 45 min, the experimental animals were injected with melatonin, whereas the control animals received the solvent only. Blood samples were withdrawn just prior to glucose loading and at intervals of ½, 1, 1½, 2, 3, 4 and 5 hr. Blood glucose estimation was done and the results statistically analysed and evaluated.

Influence on blood glucose circadian rhythm : The animals were housed in a well-lighted and ventilated room. No artificial light was used and the animals were kept in natural light conditions. The animals were subdivided into two groups of 10 each for fasting and feeding. The same ten animals in each group served as both control and experimental, to avoid any possible variations in experimental results.

There are varied reports in the feeding habits of the wild and laboratory rabbits. The feedng pattern of the wild rabbits was reported (12) to be characterized by two main periods of eating at dawn and dusk. Similar habits were reported by some workers (18) in the laboratory rabbits, while others have reported the rabbits to be an intermittent eater (5, 14) consuming food throughout the day. In view of this, the feeding group of rabbits were pre-conditioned, for at least 10 days, to feeding for 2 hours between 08.00 hr and 10.00 hr. Similarly, the fasting group of animals were pre-conditioned to removal of food every day at 16.00 hr for at least 10 days, to obtain uniform fasting conditions and to avoid intermittent eating at night by the rabbits. Such pre-conditioned animals were used in all the experiments.

Following it, both groups were injected with melatonin, after collection of first blood sample at 08.00 hr. The subsequent samples were collected at four hourly intervals for the next 24 hr. The fasting group of animals were kept fasting throughout the experimental period, whereas the feeding group was given pellet food between 08.00 hr and 10.00 hr with free access to water.

The control and melatonin treatment experiments were carried out with a gap of at least 10 days in the same animals of each group to eliminate possible variations in the results due to genetic, biological and repeated vene-puncture effects.

RESULTS

Glucose tolerance after melatonin treatment :

(Control group) : The fasting blood glucose levels prior to oral glucose loading was 77.49±3.84 *mg*/100 *ml*. After oral glucose loading it rose to a peak value of 98.89±5.26 *mg*/100 *ml* by 30 min. Then the blood glucose levels started falling and attained a level of 71.43±3.32 *mg*/100 *ml* by 2 hr, thus representing a normal response to glucose loading (Table I). The time interval taken for glucose level to return to fasting value after glucose loading was observed to be 1.75±0.14 hr (Table II), (Fig. 1).

Experimental group : The fasting blood glucose level prior to oral glucose loading was 65.36±3.26 *mg*/100 *ml*. After oral glucose loading the levels rose to a peak of 121.60±6.26 *mg*/100 *ml* by one hour (Table I). It is to be noted that there is a delay in attainment of peak blood glucose levels by 30 minutes. After an hour, the blood glucose levels showed a gradual fall and attained a level of 61.20±3.95 *mg*/100 *ml* at the end of the 5th hour suggesting decreased glucose tolerance. The time interval taken for glucose level to reach the fasting levels after glucose loading was observed to be 1.75±0.14 hr for control animals as compared with 4.80±0.06 hr for experimental animals. Statistically this was highly significant ($P<0.01$ Tasle II), (Fig. 2).

Blood glucose circadian rhythm after melatonin administration :

I. *Fasting Group* (Control) : Blood glucose levels did not show any significant variations during the 24 hour period. The lowest value observed was at 16.00 hr (Table III).

Experimental : Melatonin shifted the minimum value from 16.00 hr to 04.00 hr (next day). with another significant fall at 20.00 hr. All the levels except at 04.00 hr and 20.00 hr were significantly higher than control values (Table IV).

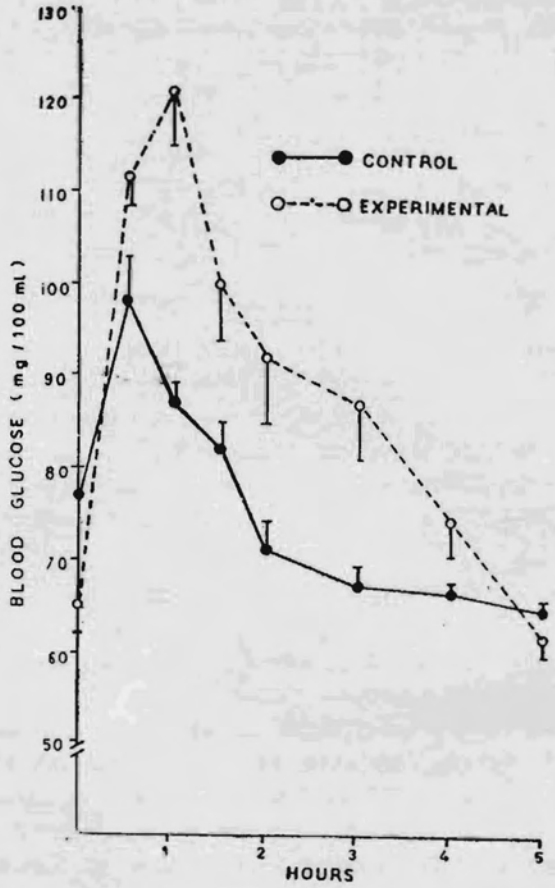

Fig. 1 : Glucose tolerance in control and experimental animals.

II. *Feeding Group* (Control) *;* The blood glucose levels in feeding control animals showed significant variation. Lowest level was at 16.00 hr with peaks at 12.00 hr and 04.00 hr (Table V).

Experimental : Melatonin shifted the minimum values by 4 hours from 16.00 hr to 20.00 hr. At 08.00, 12.00 and 16.00 hr the rise was significant compared to control feeding animals (Table VI).

From the above observations it may be noted that :

TABLE I : Blood glucose levels (Mean±S.E.) in control and experimental animals after glucose loading.

	Time interval (in hours)							
	0	½	1	1½	2	3	4	5
Control (10)	77.49 ±3.84	98.89 ±5.26	87.96 ±2.16	82.66 ±3.08	71.43 ±3.32	67.06 ±2.77	66.41 ±1.84	64.41 ±1.91
Experimental (10)	65.36 ±3.26	112.32 ±4.90	121.20 ±6.26	100.03 ±6.29	92.11 ±7.02	87.63 ±6.01	74.84 ±4.03	61.20 ±3.95
P	<0.05	>0.11	<0.01	<0.05	<0.05	<0.02	>0.1	>0.5

Number of animals in parenthesis.

TABLE II : Time interval (in hours) for attaining the initial fasting level in control and experimental animals after glucose loading.

	Hours (Mean±S.E.)	*P*
Control (10)	1.75±0.14	<0.01
Experimental (10)	4.80±0.06	

Number of animals in parenthesis.

TABLE III : Circadian rhythm of blood glucose in fasting control rabbits (n=10).

	Time interval (in hours)						
	08.00	12.00	16.00	20.00	00.00	04.00	08.00
Mean	62.27	60.26	59.67	67.63	67.79	72.30	77.85
S.E.	4.95	5.16	5.14	3 71	4.22	3.92	2.69

08.00 hr level vs 16.00 hr level P>0 5
16.00 hr level vs 08.00 hr level P<0.01

TABLE IV : Circadian rhythm of blood glucose in fasting melatonin treated rabbits (n=10).

	Time interval (in hours)						
	08.00	12.00	16.00	20.00	00.00	04.00	08.00
Mean	72.91	76.52	85.76	67.60	83 50	65.70	69.96
S.E.	4.54	5.64	5.41	4.48	6.60	6.80	5.87

16.00 hr level of control vs 16.00 hr level of melatonin treated P<0.01.

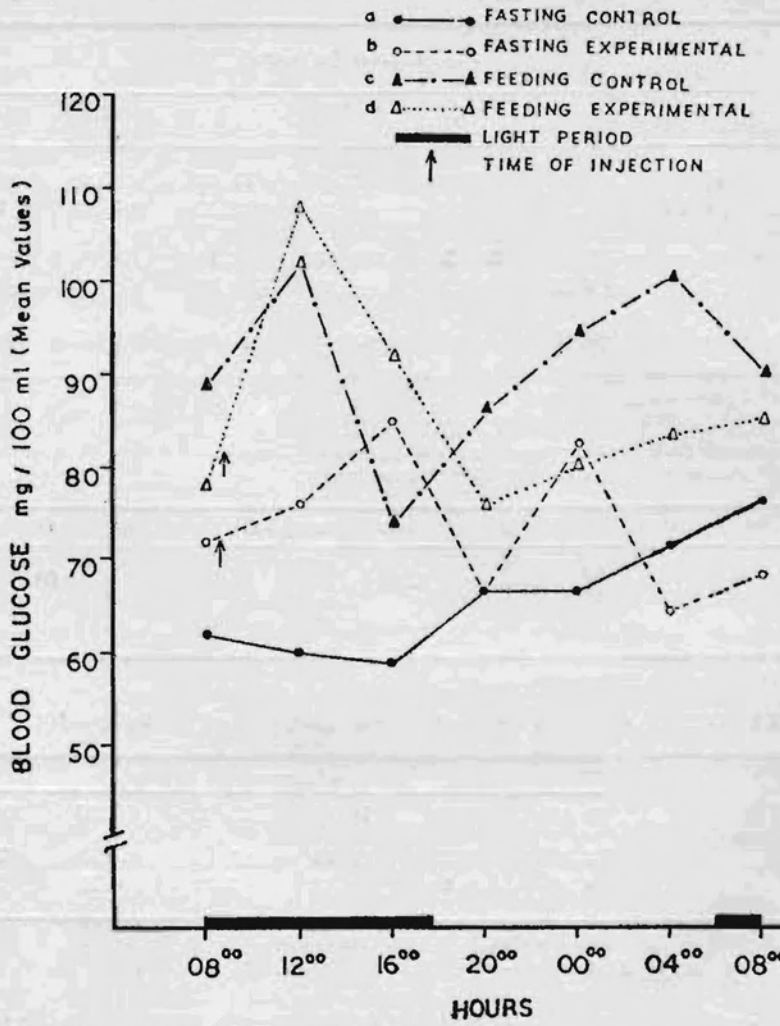

Fig. 2 : Circadian rhythm of blood glucose in fasting and feeding groups.

1. The blood glucose levels in control rabbits showed a circadian rhythm which was monophasic in fasting group and biphasic in feeding group (Fig. 2).

2. The minimum levels of blood glucose was observed at 16.00 hr in both the fasting control and feeding control groups.

3. Melatonin treatment lead to a disturbance in the normal circadian rhythm of blood glucose levels in fasting groups and caused a shift in the time of occurrence of minimum levels.

TABLE V : Circadian rhythm of blood glucose in feeding control rabbits (n=10).

	Time interval (in hours)						
	08.00	12.00	16.00	20.00	00.00	04.00	08.00
Mean	89.50	102.54	74.77	87.64	95.27	101.69	91.18
S.E.	2.75	8.12	2.44	4.13	3.38	3.47	2.57

08.00 hr level vs 12.00 hr level $P<0.5$
12.00 hr level vs 16.00 hr level $P<0.01$
16.00 hr level vs 04.00 hr level $P<0.05$

TABLE VI : Circadian rhythm of blood glucose in feeding, melatonin treated rabbits (n=10).

	Time interval (in hours)						
	08.00	12.00	16.00	20.00	00.00	04.00	08.00
Mean	78.17	108.09	92.56	76.1	81.27	84.77	86.08
S.E.	2.12	4.75	2.86	1.33	2.54	3.27	3.11

16.00 hr level of control vs 16.00 hr level of melatonin treated $P<0.01$
08.00 hr level vs 12.00 hr level $P<0.01$
12.00 hr leve vs 20.00 hr level $P<0.01$

DISCUSSION

Glucose tolerance is commonly employed to assess the functional status of the *B* cells of islets of Langerhans. Decreased glucose tolerance indicates a functional defect in these cells and may denote decreased insulin secretion by the *B* cells. In the present study melatonin injection after oral glucose loading, lead to decreased glucose tolerance, as shown by the increased time interval taken to attain initial fasting glucose levels, indicating a suppressive effect on insulin secretion.

Using *in vitro* studies, Bailey *et al.* (1) have indicated that melatonin suppresses glucose induced insulin secretion. Others (2) have reported hyperglycaemia after blinding which stimulates melatonin secretion. Hyperglycaemia folowing melatonin

injection is also reported (3). The present findings support the results of previous workers. Melatonin has reduced glucose tolerance probably by suppressing glucose induced secretion of insulin from the islet cells. Our histological studies (unpublished data) showing absence of granules in *B* cells of Langerhans after melatonin treatment, support the statement.

Only Milcu (13) has reported increased glucose tolerance and hypoglycaemia as an effect of melatonin. However, he administered a peptide extract of bovine pineal gland. Probably this peptide extract was melatonin free. (Melatonin is an indole derived from serotonin). The increased glucose tolerance reported by Milcu could be due to some as yet unknown peptide in the pineal gland.

In his observations on the blood glucose circadian rhythm in rabbits fed *ad libitum*, Eleftheriou (8) recorded minimum levels at 14.00 hr. Chakrabarty (4) observed minimum levels at 16.00 hr in tropical climatic conditions using *ad libitum* fed rabbits. Others (10) observed lowest levels around 12.00 hr in the serum glucose of rabbits. The time of attainment of minimum levels of glucose observed in the present study is in close agreement with that of Chakrabarty (4). The difference in time in the occurrence of minimum levels observed in the present study and that by other workers may be explained on the basis of different climatic conditions or species variation. Feeding probably might not be an important contributing factor as is evidenced by the occurrence of minimum levels at 16.00 hr in both feeding and fasting control groups and by the observations of Chakrabarty using rabbits fed *ad libitum*.

A circadian rhythm of serum immuno-reactive insulin (IRI) in mice has been reported (11). The IRI levels start rising at 16.00 hr, falling to a minimum at 04.00 hr. The results of the present study on blood glucose circadian rhythm can be extrapolated on these studies, the blood glucose levels at 08.00 hr being higher (IRI level lower) and that at 16.00 hr being lowest (IRI level highest), reaching higher levels again at 04.00 hr (IRI level lower). Though rabbits were used in the present study, it is closely related to mice, the animals used for the earlier studies on circadian rhythm of immunoreactive insulin.

The shift in the minimum level observed in the experimental groups (fasting and feeding) may be due to suppression of insulin released by melatonin, which is known to reduce glucose tolerance (1,17).

The present findings have revealed that melatonin caused a disturbance in the circadian rhythm of blood glucose in the fasting and feeding rabbits and caused a

shift in the time of occurrence of minimum levels. The rational explanation for the above finding might possibly be due to the action of melatonin on the islets of Langerhans modifying the secretion of insulin, which plays an important role in glucose homeostasis. Our own unpublished histological observations have suggested that chronic melatonin administration led to depletion of the *B* cell granules.

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