CHRONIC MELATONIN APPLICATION MODIFIES THE CYTOARCHITECTURE OF TESTIS AND DECREASES THE SPERM NUMBER IN WISTAR RAT

Gutiérrez-Ruiz J, Granados-Canseco F, Martínez-Esparza C,
Velázquez-Paniagua M, and Prieto-Gómez B.

Facultad de Medicina, UNAM, México D.F. 04510

Summary

Melatonin, a pineal hormone has an important role in seasonal reproduction. This indol seems to act at hypothalamus, pituitary gland, and/or gonad tissue level. Nevertheless the exact site of its action is not absolutely clear. The objective of this work is to determine the effect of the chronic application of melatonin in the testicular cytoarchitecture and on the spermatozoa number in the Wistar rat, a nonstational mammal. Transversal sections of the seminiferous tubules from the control rats exhibited an ovoid form with almost similar horizontal and perpendicular axis mean while, the melatonin rats these tubules exhibited a form that resembled a "sausage" and both axis were larger than those in the control animals. Moreover, the melatonin treated rats had a lower number of spermatozoa and a higher number of Leydig cells than control rats. Data indicates that melatonin elicits significant changes in the seminiferous tubules architecture and decreases the number of spermatozoids which could reduce fertility in the Wistar rat.

Key words: melatonin, cytoarchitecture, testis, spermatozoa, Leydig cells, rat.

The involvement of central indolamines in the control of mammalian reproduction has been documented by several authors. Particularly serotonin and melatonin (1, 2, 3, 4). Melatonin has been implicated in the control of neuroendocrine-gonadal activity in seasonal breeding mammals, in response to changes in day length (5). Several data indicates that target sites of melatonin action on the reproductive system could be the hypothalamus, the hypophysis, the gonads, the reproductive tract and the male-accessory genital organs. In spite of data suggesting that melatonin and the reproductive hormones are interrelated in rats as much as in humans, both nonstational species, the exact sites of this indol action in the reproductive system are not clear.

At the present time, melatonin is being used in clinics to reduce some symptoms of neurodegenerative diseases. Even on children, it is used for psychiatric problems like autism. However, the effects of long-term melatonin application are still not known. Therefore, this study was conducted to determine the effects of the chronic application of melatonin on cytoarchitecture of the testis and the spermatozoa production on male Wistar rats.

Methods

Animals

Wistar male rats weighing 35 ± 5 g were kept in a noise-isolated room with controlled temperature (25 ± 2ºC) and light (12:12 h light/dark cycle), allowing free access to laboratory chow and tap water. Animals were randomly assigned to a control or a melatonin treatment group. They were daily injected at the same hour, for seven days/week, during ten months.

Chemicals

Melatonin was purchased from Sigma Co (M-5250, mol, wt.232.3) and polyethylene glycol 200 was purchased from Fluka (88440). Melatonin was dissolved in polyethylene glycol at 1%.

Experimental protocol

Rats were divided into two groups. The control group (6 rats) received polyethylene glycol (1%, melatonin's vehicle) injections, while experimental group (6 rats) was given melatonin injections (1mg/kg body weight). The i.p. injections were applied for a ten-month period. Animals were sacrificed 24 hours after the last injection and the testis were isolated, weighed and stored in formalin at 10% for histological examination.

Testis were cut (7µm), processed and stained with haematoxylin-eosin for histological studies. Under microscope, one hundred and eighty samples of 10X10cm per group were analyzed for counting spermatozoa and Leydig cells. Twenty four seminiferous tubules were selected at random (12 melatonin, 12 control) and using a graduated grid the horizontal and vertical axis were measured.

Statistical analysis

The values of spermatozoa and cells of Leydig are the result of the means of 180 testicular transversal sections and twelve seminiferous tubules by group. All data are expressed as mean with standard error. The groups were compared with t Student's test at $p<0.001$

Results

There were no significant differences ($P > 0.01$) in the mean testis weight of melatonin treated rats, compared to the vehicle-treated control group. However, there were significant differences in the testicular cytoarchitecture; the control rats exhibited seminiferous tubules with an ovoid shape and a vertical axis of 292.50 ± 5.76µm and horizontal axis of 236.25 ± 10.7 µm meanwhile the melatonin-treated rats exhibited tubules in a "sausage" shape with a significant bigger vertical axis (508.75 ± 30.20 µm) than control rats, as the horizontal axis (291.25 ± 19.38 µm) does not have significant differences with the control tubules.Figures 2 and 3. In control rats the interstitial space between the tubules is observed with few cells whereas in melatonin rats these spaces are bulky with Leydig cells. Fig1

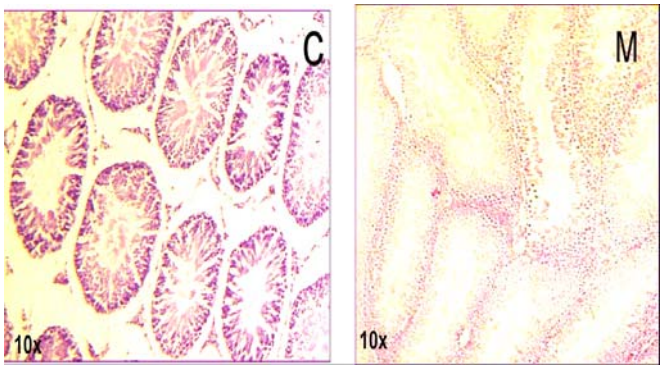

Fig.1.Photography shows a transversal section of testis in which seminiferous tubules from control and melatonin rats are observed. Hematoxylin-eosin.10X

There was a significant decrease ($p<0.001$) in sperm count of the melatonin group when compared to the vehicle-treated rats. The control average of spermatozoa of 98.73 ± 2.23, while the melatonin-rats average was of 78.6 ± 5.55. Figure 4

There was a significant increase ($p<0.001$) in the number of the Leydig cells from melatonin-treated animals compared to the control rats. The control average value of Leydig cells was 55.33±3.8 whereas the value of the rats treated with melatonin was 126±6.3. Figure 5

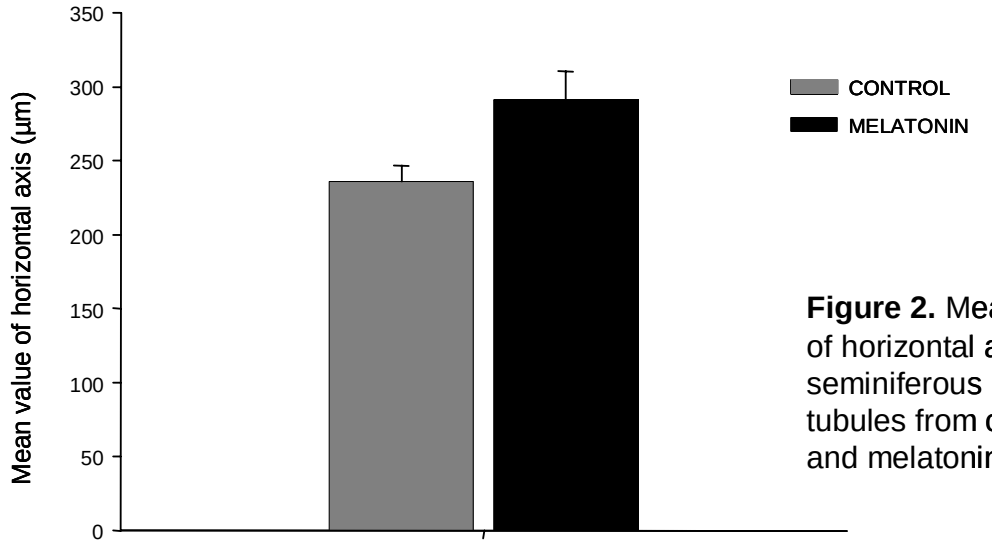

Figure 2. Mean value of horizontal axis of seminiferous tubules from control and melatonin rats.

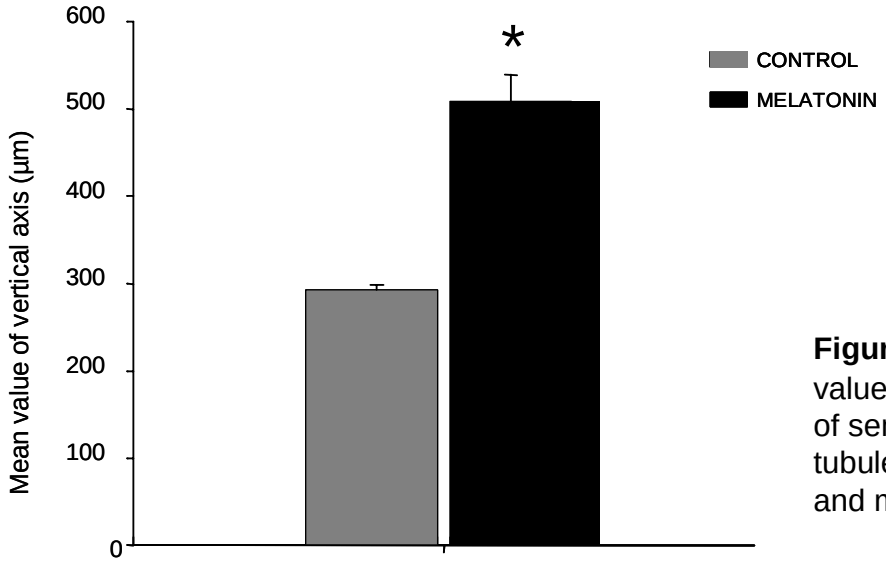

Figure 3. Mean value of vertical axis of seminiferous tubules from control and melatonin rats

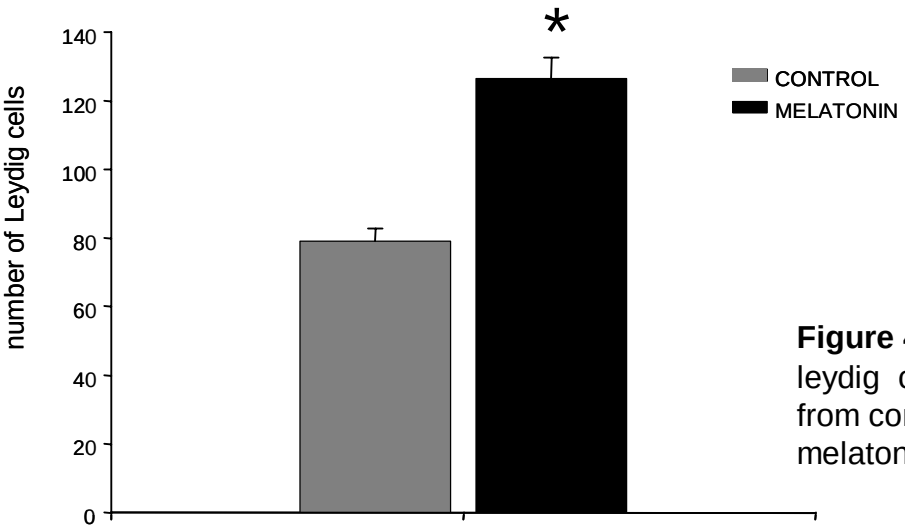

Figure 4. Mean of leydig cells number from control and melatonin rats

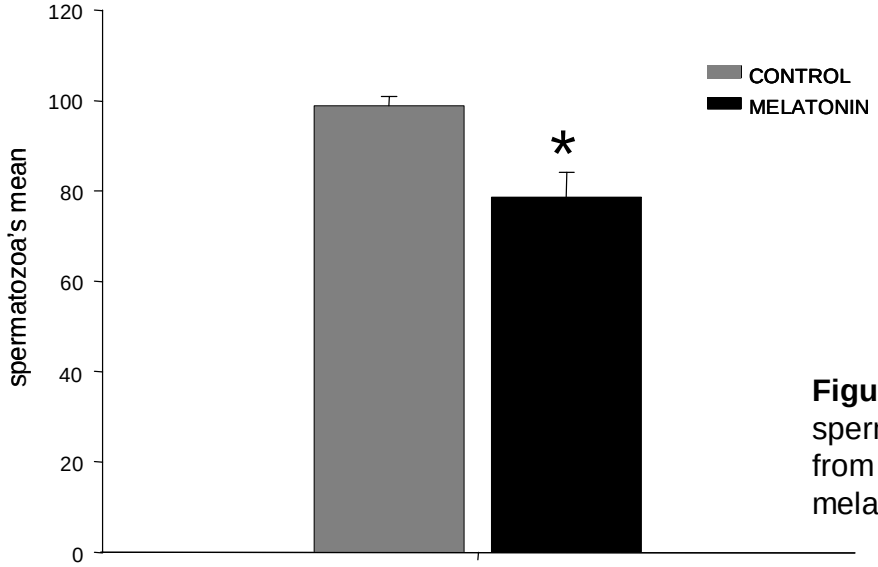

Figure 5. Mean of spermatozoa number from control and melatonin rats

Discussion

The results of the present study suggested that melatonin has an adverse effect on male reproductive apparatus in rats. Melatonin did not cause a significant decrease in testis weight but it did change the seminiferous tubules cytoarchitecture, the spermatozoa and the Leydig cells quantity. Although the mechanisms by which melatonin modified the reproductive function are not well established, this indol may affect the hypothalamic-pituitary-gonadal axis. One way in which melatonin can decrease the spermatogenesis observed in this work is by reducing the transcription of GnRH (6) or by decreasing the binding of gonadotropin-releasing hormone (GnRH) in the pituitary and lowering the pituitary gonadotropin contents (7). Also melatonin may change the spermatozoa amount by directly affecting the spermatozoa. Luboshitzky et al, (8) had reported changes in the sperm amount in rats, suggesting that this effect could be the result of inhibiting testicular aromatase, modifying the androgen: estrogen balance. Any of these mechanisms can generate a significant decrease in the number of spermatozoa. We did not analyze the melatonin's effect on sperm motility buy Gwayi (9) already reported a decrease in the sperm motility in vitro with the application of melatonin. Even though the mechanism is not very clear, it is postulated (10) that melatonin inhibition might proceed by acting on sperm membranes or by a modification on the tubulin of the sperm flagellum to decrease motility.

The possible acute action of melatonin on the rat Leydig cells is an inhibition of the steroidogenesis as suggested by Valenti 1995 (11). However, in our work the number of these cells is significantly increased. This effect could be the result of a Leydig cell-hypersensitivity to LH (even thought LH can be decreased in our work) by a decrease in the number and affinity of the melatonin receptors in the Leydig cells as seen by the prolonged use of melatonin (12), nonetheless, it seems that this hypersensitivity increases the Leydig cells number even though it is not sufficient to support the spermatogenesis. Furthermore, there are many other paracrine and autocrine factors that could be modulating the number of Leydig cells to compensate the lack of endocrine modulation elicited by melatonin. Amongst these multiple factors, there are growth factors (IGF-I), and a GnRH-like growth factor (13), which can ultimately stimulate the multiplication of Leydig cells.

The significant increase in the axis of the seminiferous tubules indicates that they grew even if the levels of gonadotropins dependent of GnRH were probably decreased. It has been observed that there are regressive changes in the seminiferous tubules with melatonin application (14), nevertheless, in this work the treatment lasted so long that the gonads could have hormonal hypersensitivity as it was mentioned before.

Melatonin has been used to treat diverse organics alterations, from jet lag to neurodegenerative diseases like Alzheimer (15). In autistic children (16) and other paediatric sleep disorders (17), melatonin is used as a sleep-promoting agent. On the other hand the dosage and the time in which it is used are very variable. Dosages have been used from 0.5mg to 50 mg/day by days or years. To the light of the data collected in this work, and although it is not possible to translate these results to man, the possible reproductive effects must be considered when using this hormone during long time in young humans.

References

1. Lecorre S, Chemineau P. Serotoninergic 5-HT(2) receptors mediate the inhibitory action of serotonin on luteinizing hormone secretion in ovariectomized, estradiol-treated ewes that are refractory to short days. Biol Reprod 1993; 49:140–147.

2. Vitale ML, Chiochio SR. Serotonin, a neurotransmitter involved in the regulation of luteinizing hormone release. Endocr Rev 1993; 14: 622–641.
3. Reiter RJ. The pineal gland and its hormones in the control of reproduction in mammals. Endocr Rev 1980; 1: 109–131.
4. Arendt J. Role of the pineal gland and melatonin in seasonal reproductive function in mammals. Rev of Reprod Biol 1986; 8:266–320.
5. J Arendt. Melatonin and the pineal gland: influence on mammalian seasonal and circadianphysiology. Rev Reprod 1998; 4: 13-22.
6. Roy D, Angelini NL, Fujieda H, Brown GM. Cyclical regulation of GnRH gene expression in GT1-7 GnRH-secreting neurons by melatonin. Endocrinology 2001; 142:4711-4720.
7. Rivest RW, Jaconi ME, Gruaz N, Sizonenko PC, Aubert ML. Short-term and long-term effects of melatonin on GnRH-stimulated gonadotropin secretion in pituitaries of sexually maturing rats. Neuroendocrinology. 1987; 46: 379-86.
8. Luboshitzky R, Zilla SO, Nave RL, Peretz L. Melatonin Administration Alters Semen Quality in Healthy Men. J Androl 2002; 4:572-578.
9. Gwayi N, Bernard RTF. The effects of melatonin on sperm motility in vitro in Wistar rats. Andrologia 2002; 34:391-396.
10. Irez TO, Senol J, Alagoz M, Basmaciogullari C, Turan F, Kuru D, Ertungealp E. Effects of indolamines on sperm motility in vitro. Hum Reprod 1992; 7:987-990.
11. Valenti S, Guido R, Giusti M, Giordano G. In vitro acute and prolonged effects of melatonin on purified rat Leydig cell steroidogenesis and adenosine 3',5' monophosphate production. Endocrinology 1995;136: 5357-5362.
12. Valenti S, Fazzuoli L, Giordano G, Giusti M. Changes in binding of iodomelatonin to membranes of Leydig cells and steroidogenesis after prolonged in vitro exposure to melatonin. Int J Androl 2001;24: 80-86.
13. Saez J. Leydig cells: endocrine, paracrine and autocrine regulation. Endocr Rev 1994; 15: 574-626.
14. Ng TB, Ooi VE. Effect of pineal indoles on testicular histology of mice. Arch Androl 1990;25: 137-45.
15. Ying-Hui Wu, Dick F S. The human pineal gland and melatonin in aging and Alzheimer's disease. J Pineal Res 2005; 38:141-145.
16. Panksepp J, Melatonin - The Sleep Master.An emerging role for this over-the-counter supplement in the treatment of autism. http://autism.org/melatonin.html.
17. Jan JE, O'Donnell ME. Use of melatonin in the treatment of paediatric sleep disorders. J. Pineal Res 1996;21: 193-199.