Effects of Melatonin on Reproductive and Accessory Reproductive Organs in Male Rats

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Effects of daily injections of melatonin on male rat reproductive and accessory reproductive organs were studied. The weight of the prostate was decreased by treatment with a high dose of melatonin (8.0 mg/kg, s.c., injection time; 17:00) once daily for 30 d. The weights of the other accessory reproductive organs and testes were not influenced by melatonin. Lower doses (0.8, 2.4, 4.8 mg/kg), had no effect. After the successive treatment with melatonin (8.0 mg/kg), the testosterone levels in testes and in serum and the conversion rate of [^{3}H]testosterone to [^{3}H]dihydrotestosterone in the prostate were not influenced. The activity of acid phosphatase and the uptake of [^{3}H]testosterone by the prostate, in contrast, were significantly decreased after the successive treatment with melatonin. These data suggest that melatonin may have a direct inhibitory action on male rat prostate, though only at a high dose.

Keywords melatonin; gonadal size; testosterone; male rat

Introduction

Melatonin is one of several hormones secreted by the pineal gland[1] that has been shown to influence reproductive function.[2,3] A number of investigators[4,5] have shown that melatonin has inhibitory effects on the gonads of mammals. However, recent data,[6,7] do not confirm these earlier reports. The present study was carried out to further examine the effect of melatonin on male rat reproductive and accessory reproductive organs and to relate the effect with the dynamics of testosterone in the prostate.

Materials and Methods

Male Wistar rats (Saitama Experimental Animals Supply Co., Ltd.) weighing 230—260 g were used in this study. Rats were housed five to a cage (L 55.0 × W 40.5 × H 19.0 cm) in a temperature- and humidity-controlled room (23±1 °C, 53±5%) with a 12 h light cycle (lights on 6:30—off 18:30) and fed Oriental rat pellets and tap water *ad libitum*. Melatonin (Sigma Chemical Company) was dissolved in ethanol and further diluted with 0.9% NaCl solution. The final solution contained 1.0% ethanol and the injected volume per rat was 0.1 ml containing the calculated dose of melatonin. Eight-tenths, 2.4, 4.8 and 8.0 mg/kg of melatonin was injected subcutaneously once daily for 30 d in the evening (17:00). In experiments with melatonin, the time of day is an important factor for administration: injection during the evening is effective whereas no effect is seen with morning administration.[8,9] Control rats were given vehicle alone. At 16 h after the final melatonin injection, rats were killed by decapitation and the testes and accessory reproductive organs were removed and weighed. Trunk blood was collected, allowed to clot at room temperature during an approximately 15-min period, and centrifuged at 2800 rpm for 15 min at 4 °C to collect the serum. The serum was then frozen at −20 °C for future testosterone assay.

Determination of Acid Phosphatase and Testosterone The activity of acid phosphatase was determined in the prostate by the phenyl phosphate method of Kind and King.[10] The amount of protein was determined by the method of Lowry *et al.*[11] Determination of testosterone in testis and in serum were carried out by high performance liquid chromatography (HPLC)[12] and radioimmunoassay (SRL Laboratory, Tokyo), respectively.

Uptake of [^{3}H]Testosterone and Production of [^{3}H]Dihydrotestosterone A segment of melatonin-treated prostate was incubated with Krebs Ringer bicarbonate buffer including 50 ng of [^{3}H]testosterone (5 ng/ml, 103 Ci/mmol, Amersham) for 15—30 min at 37 °C under a mixed gas stream of O_2–CO_2 (95:5). After incubation and washing, the prostate was homogenized with 90% ethanol solution; the layer of ethanol solution was washed with petroleum ether and was evaporated. The residue was applied to a thin layer plate of silica gel (solvent system, cyclohexane–ethylacetate, 6:4 v/v). The identification of testosterone and of dihydrotestosterone on the thin layer chromatography (TLC) plate was carried out Ultraviolet (UV) lamp and I_2 vapor, respectively; radioactivity of each spot was measured in a liquid scintillation counter.

Statistical Analysis The statistical significance of differences between groups was assessed by Student's *t*-test.

Results

Successive treatment with melatonin had no effect on the testis weight, epididymis, *vas deferens* or seminal vesicle in male rats. However, the weight of the prostate was significantly decreased in the melatonin (8.0 mg/kg)-treated rats (Table I). Eight-tenths, 2.4 and 4.8 mg/kg of melatonin dose had no effect on testes and accessory reproductive organs. The testosterone level in testis and in serum were not affected by the treatment with melatonin (8.0 mg/kg)

TABLE I. Influence of Daily Injections of Melatonin on Gonadal Size in Male Rats

Dose (mg/kg)	Testis	Epididymis	*Vas deferens*	Seminal vesicle	Prostate
0.0	1276±264	524±76.4	148±18.7	202± 3.6	248±12.5
0.8	1297±234	597±98.1	151±24.8	223±12.5	257±13.8
2.4	1301±114	583±87.9	149±11.6	219± 6.8	232±10.8
4.8	1292±204	506±45.6	129±10.6	200± 5.5	198±18.7
8.0	1206±163	527±30.6	135± 8.6	192± 7.8	182± 3.9[a]

Values are mg organ weight and the mean±S.E. of seven rats. *a*) $p<0.01$ *vs.* vehicle.

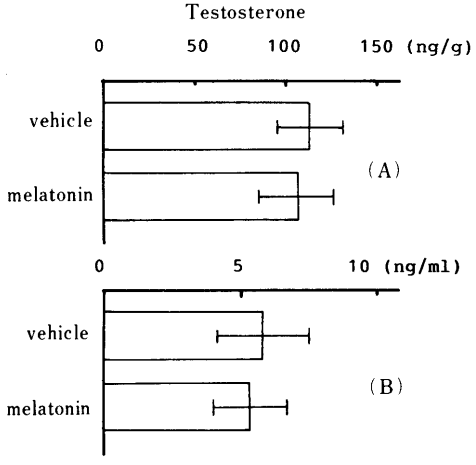

Fig. 1. Effect of Melatonin on Testosterone Levels in Testis (A) and in Serum (B)

Testis and serum were obtained from melatonin (8.0 mg/kg)-treated rats or vehicle-treated rats. Each value represents the mean±S.E. obtained from seven rats. Vehicle: 1.0% ethanol.

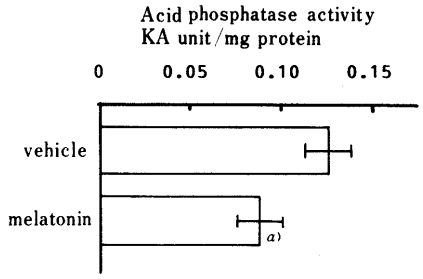

Fig. 2. Effect of Melatonin on Acid Phosphatase in Rat Prostate

Prostate was obtained from melatonin (8.0 mg/kg)-treated or vehicle-treated rats. Each value represents the mean ± S.E. obtained from seven rats. Vehicle: 1.0% ethanol. KA unit: Karmen unit. *a*) $p<0.05$ *vs.* vehicle.

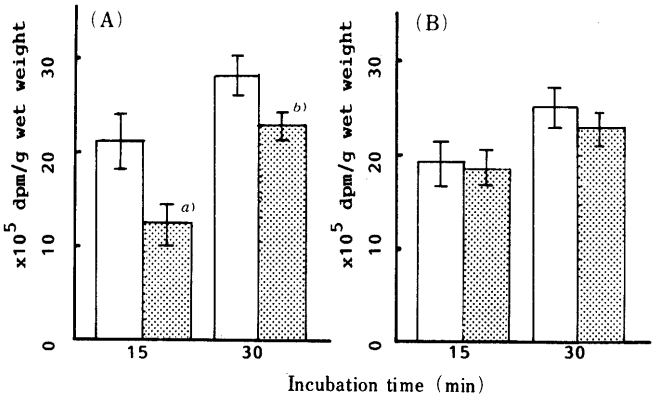

Fig. 3. Effect of Melatonin on Uptake of [^{3}H]Testosterone (A) and Production of [^{3}H]Dihydrotestosterone (B) in Prostate

Values are the mean and S.E. obtained from seven prostates. Open column: vehicle-treated rat prostate. Dotted column: melatonin (8.0 mg/kg)-treated rat prostate. *a*) $p<0.01$ *vs.* vehicle. *b*) $p<0.05$ *vs.* vehicle.

(Fig. 1). Acid phosphatase in the melatonin (8.0 mg/kg)-treated rat prostate was decreased compared with the vehicle-treated rat prostate (Fig. 2). On the other hand, the uptake of [^{3}H]testosterone into the melatonin (8.0 mg/kg)-treated rat prostate was significantly decreased compared with control prostate at 15 and 30 min of incubation time. However, the conversion of [^{3}H]testosterone to [^{3}H]dihydrotestosterone was not observed in the melatonin (8.0 mg/kg)-treated rat prostate (Fig. 3).

Discussion

The results of the present study show that successive treatments of a high dose of melatonin (8.0 mg/kg, 1/100th the toxic dose)[13] to male rats resulted in a significant decrease in the prostate weight, acid phosphatase activity and the uptake of [^{3}H]testosterone in the prostate. It seems that melatonin dose used in this experiment induces the pharmacological effect rather than the physiological action.

Several previous studies indicate that melatonin functions as a hormone in mammals since it delays vaginal opening and disrupts the first estrous cycle in immature female rats, and reduces seminal vesicle weight in mature male rats.[14] Other investigators suggest that the effect of melatonin may be due, as Ellis[15] observed in the testes, to its direct action on the gonads, or through the inhibition of secretion of gonadotropines from the hypophysis. There have also been reports of a lack of effect of melatonin and of an increase in ovarian weight after melatonin treatment. These reports[16,17] hence do not yet permit an understanding of the role of melatonin in the female rat or, for similar reasons, in the male rat.

In the present investigation, the testosterone levels were not influenced by successive melatonin treatment; it is considered that melatonin would not act by modifying the release of gonadotropin from the hypophysis. These results are supported by those of Debeljuk *et al.*[18] Who found that melatonin did not effect pituitary or serum luteinizing hormone (LH) concentration. On the other hand, Shirama *et al.*[14] reported that melatonin induced the increase of prolactin and dihydrotestosterone levels in serum of pinealectomized male rats. This report suggested that the raised prolactin levels may also be responsible for the interference in the action of hormones in androgen-dependent tissue.

The acid phosphatase level in the prostate has been considered an index of testosterone function.[19] In melatonin-treated rats (Fig. 2), the acid phosphatase activity in the prostate was decreased compared with control, suggesting that melatonin may have an inhibitory action to protein synthesis[20] in the prostate through the decrease of testosterone uptake. On the other hand, melatonin has been shown to influence the steroid metabolizing enzymes in serveral endocrine organs.[21] In the prostate from melatonin-treated rats, the uptake of [^{3}H]testosterone was depressed. These results appear to indicate that melatonin may have a direct action on the prostate in rats. Additionally, the action of melatonin on the conversion of testosterone to dihydrotestosterone in the prostate was not observed in this experiment. Moreover, Shirama *et al.*[22] obtained evidence that melatonin can act at the tissue level to prevent the conversion of testosterone to dihydrotestosterone. Horst and Adam[23] noted that melatonin did not influence the activity of 5α-reductase in the rat prostate. Thus, the data in the present experiments suggest a possible direct inhibitory action of melatonin on the prostate in male rats.

References

1) R. J. Reiter, *Endocr. Rev.*, **1**, 109 (1980).
2) L. Y. Johnson, M. K. Vaughan, B. A. Richardson, L. J. Petterborg and R. J. Reiter, *Proc. Soc. Exp. Biol. Med.*, **169**, 416 (1982).
3) K. Yamada, Y. Sakamoto and T. Satoh, *Res. Commun. Chem. Pathol. Pharmacol.*, **46**, 283 (1984).
4) L. Debeljuk, V. M. Feder and O. A. Paulucci, *J. Reprod. Fert.*, **21**, 363 (1970).
5) R. J. Reiter, *Neuroendocrinology*, **2**, 139 (1967).
6) P. Lissoni, M. Resentini, R. Mauri, C. DeMedici, F. Morabito, D. Esposti, L. DiBella, G. Esposti, D. Rossi, L. Paravicini, G. Legname and F. Fraschini, *Acta Endocr., Copenh.*, **111**, 305 (1986).
7) F. Waldhauser, H. R. Lieberman, H. J. Lynch, M. Waldhauser, K. Herkner, H. Frisch, H. Vierhapper, W. Waldhausl, M. Schemper, R. J. Wurtman and W. F. Crowley, *Neuroendocrinology*, **46**, 125 (1987).
8) F. W. Richard, M. C. Susan and S. T. Paola, *Neuroendocrinology*, **35**, 37 (1982).
9) L. Tamarkin, W. K. Westrom, A. I. Hamill and B. D. Goldman, *Endocrinology*, **99**, 1534 (1976).
10) P. R. M. Kind and E. J. King, *J. Clin. Path.*, **7**, 322 (1954).
11) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
12) K. Yamada and Y. Aizawa, *J. Pharmacol. Methods*, **10**, 164 (1983).
13) J. Barchas, F. DaCosta and S. Spector, *Nature* (London), **214**, 919 (1967).
14) K. Shirama, T. Furuya, Y. Takeo, K. Shimizu and T. Maekawa, *J.*

Endocrinol., **95**, 87 (1982).
15) L. Ellis, *J. Reprod. Fert.*, **18**, 159 (1969).
16) T. M. John, J. C. George and R. J. Etches, *J. Pineal Res.*, **3**, 169 (1986).
17) W. C. Adams and W. L. Sohler, *J. Endocrinol.*, **31**, 295 (1965).
18) L. Debeljuk, V. M. Feder and O. A. Paulucci, *J. Reprod. Fert.*, **21**, 363 (1970).
19) R. J. Ablin, *Clin. Chem.*, **19**, 786 (1973).
20) J. R. Kent, B. V. Suryanaryana and M. Hill, *Invest. Urol.*, **7**, 250 (1969).
21) H.-J. Horst, A. Buck and K.-U. Adam, *Experientia*, **38**, 968 (1982).
22) K. Shirama, T. Furuya, Y. Takeo, K. Shimizu and K. Maekawa, *J. Endocrinological Invest.*, **4**, 203 (1981).
23) H.-J. Horst and K.-U. Adam, *Horm. Metabol. Res.*, **14**, 54 (1982).