Influence of Melatonin on Reproductive Behavior in Male Rats

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The influence of melatonin on reproductive behavior was examined by administering it to pinealectomized male rats for 30 consecutive days. After the 30-d administration of melatonin or vehicle, each male rat was introudced into a female rat's cage on the day of proestrus and allowed to mate overnight. All rats administered the vehicle and the pinealectomized rats copulated; five of the twelve melatonin (8.0 mg/kg)-treated rats did not copulate. These findings suggest that melatonin inhibits the reproductive behavior of male rats.

Keywords melatonin; reproductive behavior; male rat; copulation

Introduction

It has been demonstrated that melatonin is one of several hormones produced in the pineal gland, which is known to produce hormones having antigonadal activity.[1-3] For example, melatonin given daily in the afternoon induced testicular and accessory reproductive organ regression in male animals and stopped estrous rhythmicity in female animals.[4] In the field of reproductive behavior in some birds[5] and lower vertebrates[6] it is an important component of a coupling agent. There is, however, no information concerning the effects of melatonin on the reproductive behavior of male rats. In the present study, we examined the reproductive behavior of male rats administered melatonin for 30 consecutive days.

Materials and Methods

Male Wistar rats (four weeks after birth) purchased from Saitama Experimental Animals Supply Company (Saitama Prefecture, Japan) were used. Pinealectomy was performed under ether anesthesia according to the procedure of Hoffman and Reiter.[7] After pinealectomy, rats were housed six to a cage (L. 55.0 × W. 40.5 × H. 19.0 cm) in a temperature and humidity-controlled room (22±2 °C, 53±5%) with a 12-h light cycle (lights on 6:30—off 18:30) and fed Oriental rat pellets and water *ad libitum*. After one week of habituation, the experiment was started. Forty-eight pinealectomized male rats were divided into the following four groups of twelve rats each: group 1, pinealectomy; group 2, 10% ethanol treatment (2.0 ml/kg/d, s.c.); and groups 3 and 4, melatonin (Sigma Chemical Company) dissolved in 10% ethanol (3.0, 8.0 mg/kg/d, s.c.) was injected at 17:00. In the experiments with melatonin, the time of day is an important factor in administration: injection during the evening is effective whereas no effect is seen with morning administration.[8] The dose of melatonin used (8.0 mg/kg) is 1/100 of the toxic dose.[9] Treatment was carried out in from immature to mature male rats. After being subjected to each treatment for 30 d, each male rat was mated with each healthy female Wistar-strain rat (10 weeks after birth) at the stage of proestrus. Mating was confirmed to have occurred by the presence of vaginal copulation plugs and the presence of spermatozoa in the vagina under microscopic observation. The day on which mating was confirmed was designated day 0 of gestation. On the 20 th day of gestation, dams were killed by decapitation, and the numbers of implantations, viable and dead fetuses, and resorbed embryos were counted.

Results and Discussion

When housed with mature female rats, all rats in group 1 copulated. In group 2, one, in group 3, two, and in group 4 five male rats did not copulate with female rats. The rate of pregnancy was 100% (12/12) in group 1,91.7% (11/12) in group 2, 83.3% (10/12) in group 3, and 58.3% (7/12) in group 4, the latter rate being significantly lower.

There were significant differences among the four groups in number of resorbed embryos, *i.e.*, 3 in group 1, 8 in group 2, and none in group 3 or group 4. The finding of 8 resorbed embryos in group 2 (10% ethanol-treated group) is interesting with respect to alcoholic toxicity.[10] This phenomenon suggests that ethanol administration over a long period may damage the spermatozoal desoxyribonucleic acid (DNA). Melatonin was dissolved in 10% ethanol but no resorbed embryos were observed in the melatonin-treated groups. This phenomenon is still not clearly explained.

No in utero fetal deaths were observed in either ethanol-treated group. There were no differences among the four groups in the weight of fetuses or of placenta.

Melatonin is a hormone secreted by the pineal gland known to have an inhibitory effect on the gonads in mammals.[11,12] The chronic administration of melatonin reduces the weight of the seminal vesicles and prostate of male rats.[13,14] Similar results were obtained after daily administration of melatonin in our experiment, *i.e.*, the weights of seminal vesicle and prostate were significantly decreased compared with those in vehicle-treated male rats.[15] On the other hand, high sperm motility is associ-

TABLE I. Influence of Daily Administration of Melatonin to Male Rats on Pregnancy Rate in Female Rats

Treatment of male rat	Dose	$N^{a)}$	$N^{b)}$	Pregnancy rate (%)
Pinealectomy		0	12	100.0
+10% ethanol	2 ml /kg	1	11	91.7
+Melatonin	3 mg/kg	2	10	83.3
	8 mg/kg	5	7	58.3[c)]

Twelve male and female rats were used. *a)* N; No. of non-copulated male rats. *b)* N; No. of gestated female rats. *c)* Significantly different from 10% ethanol-treated group ($p<0.01$).

TABLE II. Influence of Melatonin and Vehicle Treatment of Male Rats on Fetuses in Gestated Female Rat's Uterus

Treatment of male rats	No. of fetuses in litter	Weight of fetus (g)	Weight of placenta (g)	Fetus mortality	No. of resorbed embryos
Pinealectomy	13±1	1.92±0.09	0.34±0.01	1	3
+10% ethanol	12±2	2.02±0.11	0.34±0.02	0	8
+Melatonin					
3 mg/kg	11±3	1.96±0.08	0.35±0.01	0	0
8 mg/kg	11±1	2.11±0.12	0.36±0.01	0	0

No. of dams used for : pinealectomy, 12; 10% ethanol, 11; melatonin 3 mg/kg, 10; 8 mg/kg, 7.

ated with improved conception rates. Bornman *et al.*[16] reported that melatonin has effects similar to those of colchicine on the microtubules in the testis. Colchicine can interfere with microtubular function. However, the present findings showed that melatonin plays no important role in sperm motility. In our observation, sperm motility did not change between the vehicle group and melatonin-treated rats. Furthermore, Ellis[17] and Debeljuk *et al.*[18] pointed out that melatonin did not decrease gonadotropin levels in the pituitary gland or in the serum. Also, melatonin did not influence the testosterone levels in the blood.[15] Many other studies have demonstrated inhibitory effects of this pineal hormone on the reproductive function and system in male and female rats. Shirama *et al.*,[14] Kamberi *et al.*,[19] and Waldhauser *et al.*[20] reported that melatonin induced the increase of prolactin levels in blood. Therefore, in the melatonin-treated male rats exposed to females reproductive behavior may be curbed by suppression of the copulative appetite in the central nervous system,[21] including the influence of prolactin or by a direct inhibitory action on accessory reproductive organs. We believe that melatonin affects the regulatory area of sex behavior in the hypothalamus[22] or limbic system.

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