The Inhibition of PMSG-induced Ovulation in Immature Rats by Melatonin (35634)

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A significant body of experimental evidence is accumulating which points to a major role of the pineal gland in the control of gonadal function. For example, pinealectomy in male rats results in testicular hypertrophy and enlarged prostate glands and seminal vesicles (1), while effects in females include ovarian and hypophyseal hypertrophy, an increased incidence of estrus, and precocious puberty (2–5). These effects of pinealectomy may be reversed by the administration of pineal extracts and, in general, also by the pineal amine, melatonin (1–5). Studies of the mechanism by which the pineal gland influences the gonads are focusing on interactions between the pineal, its amine constituents, and the functioning of the hypothalamic–hypophyseal axis. The results of several investigations have demonstrated a relationship between pinealectomy and increased levels of pituitary and circulating gonadotropins, and the reversal of this effect of pinealectomy by the administration of pineal extracts or melatonin (6–9). It therefore seems likely that the pineal gland may control gonadal function through modulation of the release of hypophyseal gonadotropins, perhaps in a manner analogous to the control exerted by estrogens and progestagens over gonadotropin levels, by regulating the release of hypothalamic releasing hormones (10, 11). The evidence also suggests that melatonin may be the chemical mediator of pineal control. The results of the present study, which utilized gonadotropin-stimulated ovulation in the immature rat as a model, support these hypotheses.

Materials and Methods. Immature, 21-day-old Wistar-derived rats, obtained from Harlan Industries, Cumberland, Indiana, were used. The animals were provided Purina Laboratory Chow and water *ad libitum* and were maintained under a controlled light regimen of 12 hr of light (6 a.m.–6 p.m. CST) and 12 of darkness. On the morning after receipt (*i.e.*, at 22 days of age) each animal received, subcutaneously, 3 IU of pregnant mares serum gonadotropin (PMSG; Equinex, Ayerst Laboratories, New York, N.Y.) in 0.2 ml of saline. Two days later (24 days of age) the animals were given melatonin (Aldrich Chemical Company, Milwaukee, Wis.) in saline either orally 52 or 53 hr after PMSG, or subcutaneously 53 hr after PMSG. Additional groups were given, subcutaneously, 3 IU of human chorionic gonadotropin (HCG; A.P.L., Ayerst Laboratories, New York, N.Y.) concurrently with the melatonin. The rats were killed 19–23 hr after melatonin treatment and the oviducts were excised and flushed with a solution containing 0.5% hyaluronidase (Calbiochem, Los Angeles, Calif.) in saline. The flushings were collected in microconcavity slides and observed through a dissecting microscope for the presence of ova.

Results. The data summarized in Table I demonstrate that the subcutaneous adminstration of melatonin 53 hr after PMSG significantly reduced the incidence of ovulation. This effect was dose-related over the range of 2 to 8 mg/kg; higher doses did not result in a greater inhibition. The number of ova recovered was also reduced by all except the lowest dose of melatonin, but this reduction was significant at only the 12 mg/kg dose. The administration of 3 IU of HCG concurrently with 8 mg of melatonin/kg overcame the inhibitory effect of melatonin.

The oral administration of melatonin 53 hr after PMSG significantly reduced ovulation in only the group that received 12 mg/kg (Table II). The average number of eggs recovered was significantly reduced in only

TABLE I. Effect of Melatonin, Administered Subcutaneously on Ovulation in Immature Rats: 53 hr After PMSG.

Treatment	Dose (mg/kg)	No. of rats ovulating/total no. of rats	(%)	Av no. of eggs ± SD
Control	0	39/40	98	3.7 ± 2.1
Melatonin	2	33/40	83[a]	4.0 ± 3.5
Control	0	36/40	90	7.6 ± 8.8
Melatonin	4	28/40	70[a]	5.9 ± 6.8
Control	0	32/40	80	4.3 ± 3.5
Melatonin	8	15/40	38[a]	2.5 ± 1.8
Control + HCG[b]	0	40/40	100	6.0 ± 2.3
Melatonin + HCG[b]	8	40/40	100	6.1 ± 1.5
Control	0	32/40	80	4.9 ± 2.7
Melatonin	12	15/40	38[a]	3.0 ± 2.3[c]
Control	0	18/20	90	6.9 ± 7.1
Melatonin	16	7/20	35[a]	4.0 ± 2.8

[a] X^2, $P<.05$.
[b] 3 IU of human chorionic gonadotropin.
[c] Student's t test, $p<.05$.

the 4 mg/kg group. In contrast (Table III), the oral administration of melatonin 52 hr after PMSG significantly reduced the incidence of ovulation at dose levels of 4, 8, and 12 mg/kg. Again, the concurrent administration of HCG prevented the inhibitory effect of melatonin.

Discussion. The administration of PMSG to immature rats stimulates follicular development and subsequent ovulation. Several lines of evidence indicate that the ovulation which occurs after PMSG administration is caused by the release of a hypophyseal ovulating hormone (12–14). Pinealectomy has been shown to increase the incidence of ovulation in PMSG-treated immature rats (6), while the concomitant administration of both melatonin and HCG failed to influence PMSG-induced ovulation (15). The results of this study, which show that the administration of a single dose of melatonin at the appropriate time prevented induced ovula-

TABLE II. Effect of Melatonin, Administered Orally, on Ovulation in Immature Rats: 53 hr After PMSG.

Treatment	Dose (mg/kg)	No. of rats ovulating/total no. of rats	(%)	Av no. of eggs ± SE
Control	0	17/20	85	2.6 ± 1.6
Melatonin	2	17/20	85	3.2 ± 2.1
Control	0	15/20	75	3.2 ± 2.0
Melatonin	4	14/20	70	1.8 ± 1.3[a]
Control	0	18/20	90	5.8 ± 2.7
Melatonin	8	15/20	75	5.3 ± 3.0
Control	0	10/10	100	4.0 ± 2.4
Melatonin	12	13/20	65[b]	4.2 ± 2.4

[a] Student's t test, $p<.05$.
[b] X^2, $p<.05$.

TABLE III. Effect of Melatonin, Administered Orally, on Ovulation in Immature Rats: 52 hr After PMSG.

Treatment	Dose (mg/kg)	No. of rats ovulating/total no. of rats	(%)	Av no. of eggs ± SD
Control	0	24/30	80	4.9 ± 3.4
Melatonin	2	15/20	75	4.5 ± 2.9
	4	14/30	47[a]	5.0 ± 2.2
Control	0	14/20	70	3.5 ± 2.6
Melatonin	8	11/30	37[a]	2.7 ± 1.9
Control + HCG[b]	0	10/10	100	4.7 ± 1.8
Melatonin + HCG[b]	8	19/20	85	5.1 ± 1.8
Control	0	14/20	70	4.1 ± 1.5
Melatonin	12	9/30	30[a]	2.6 ± 2.4

[a] X^2, $p<.05$.

[b] Three IU of human chorionic gonadotropin.

tion, suggest that melatonin acts to prevent the release of an endogenous ovulating hormone. That this action of melatonin is central, *i.e.*, on the hypothalamus or pituitary, rather than a manifestation of an inhibitory effect on the ovary itself, is indicated by the ability of HCG to induce ovulation in melatonin-treated animals. The present findings offer further support to the hypotheses that (a) the pineal gland exerts control over gonadal function by regulating gonadotropin release at the hypothalamic-hypophyseal level; and (b) melatonin serves as the chemical mediator for this action of the pineal gland.

Summary. The subcutaneous or oral administration of melatonin to immature rats which had received PMSG to induce follicular growth and ovulation caused a significant reduction in the incidence of ovulation. Furthermore, the administration of HCG concurrently with melatonin overrode the inhibitory effect of melatonin. These observations support the concept that the pineal gland exerts control over reproductive functions via an inhibitory action of melatonin on the release of hypophyseal gonadotropins.

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