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Melatonin, a Pineal Substance: Effect on the Rat Ovary

Abstract. *Daily injection of microgram amounts of melatonin in rats decreased the incidence of estrus and reduced ovarian weight. Circulating melatonin was selectively taken up and retained by the ovary and pineal gland; this effect was reduced by exposure of rats to constant light. A single injection of melatonin lowered the incidence of estrus among rats exposed to constant light.*

Many observations have linked the mammalian pineal gland to gonad function. Human males with tumors which destroy the pineal gland have a high incidence of precocious puberty (*1*). Pinealectomy has resulted in an increase in ovarian weight (*2*), while pineal extracts decreased ovarian weight and produced anestrus in aged rats with spontaneous persistent estrus (*3*). When rats were exposed to constant light the ovaries became enlarged and the incidence of estrus increased; both effects are inhibited by the administration of pineal extracts (*4*). Exposure to light produced a decrease in pineal weight, serotonin content, and nucleolar size (*5*). Thus, some of the effects of light on the gonads may be mediated by the pineal gland.

Table 1. The effect of melatonin on the rat ovary. The diluent was 0.48 percent KCl and 0.18 percent NaCl. S, serotonin; M, melatonin; i.p., intraperitoneally; s.c., subcutaneously.

Treatment	Dose (mg)	Route	Ovary weight ± S.E. (mg)
Groups of 11 rats (85 to 95 g)			
Control			67.3 ± 3.6
S*	50	i.p.	71.4 ± 4.4
M	20	i.p.	42.6 ± 3.2 ($p \leq 0.001$)
Groups of 10 rats (45 to 55 g)			
Control			49.0 ± 2.8
M	1	i.p.	46.0 ± 2.6
M	10	i.p.	41.3 ± 2.0 ($p \leq 0.05$)
M	1	s.c.	39.1 ± 2.9 ($p \leq 0.05$)

* As serotonin creatinine sulfate.

Melatonin (5-methoxy-*N*-acetyltryptamine) is highly localized in the mammalian pineal gland (*6*), and small amounts also occur in peripheral nerves (*6*). An enzyme, hydroxyindole-*O*-methyl transferase, required for the synthesis of melatonin is present exclusively in the pineal gland (*7*). Very small amounts of melatonin produce a lightening effect on amphibian melanocytes (*6*), but melatonin has not been studied in that class. Recently, Baschieri *et al.* have reported that when rats were given large doses (150 μg) of melatonin daily for 10 days, the increments in thyroid-cell height and I^{131} uptake produced by methylthiouracil were diminished (*8*).

Table 2. Effect of melatonin on the estrous cycle of the rat. Animals received 0.2 ml of diluent or 20 μg of melatonin, intraperitoneally. Vaginal smears were taken daily after vaginal opening and 14 to 19 smears were taken from each animal. Results are expressed as the percentage of the total number of smears indicating estrus which were taken from each animal. The incidence of estrus in rats receiving melatonin differed significantly ($p \leq .01$) from that of animals receiving placebo.

Smears indicating estrus (%)	Rats (No.)	
	Control	Melatonin
0–20	0	3
21–40	2	5
41–60	7	3
61–80	2	0
81–100	0	0

Our experiments show that some of the effects of the pineal gland on gonad function might be mediated by melatonin. Immature female rats were given placebo, 50 μg of serotonin, or 1 to 20 μg of melatonin, intraperitoneally or subcutaneously, daily for 28 days. In rats receiving melatonin, there was a delay in spontaneous vaginal opening, and a highly significant decrease in ovarian weight (Table 1) and in the incidence of vaginal estrus (Table 2). As little as 1 μg of melatonin, administered subcutaneously, caused a significant decrease in the weight of the ovary. Larger doses of serotonin, the precursor of melatonin, produced none of the vaginal and ovarian effects but caused a significant increase in adrenal weight. Neither compound altered body or uterine weight.

Tritiated melatonin (*9*), when administered to four cats intravenously, was selectively taken up in endocrine and peripheral nervous tissues, especially in the ovary and the pineal gland. One hour after administration, the concentration of H^3-melatonin in the ovary was 5 to 25 times that in the peripheral tissues, 2 to 3 times that in the thyroid, pituitary, and adrenal glands and the peripheral nerves, and one-third that in the pineal gland.

When 100 rats were kept in constant or normal light for 4 weeks and then given H^3-melatonin intravenously, the uptake by the pineal gland and ovary was significantly reduced. Vaginal smears of these animals showed an 85 percent incidence of estrus, as compared with 50 percent in animals exposed to normal diurnal variation. When estrous rats, exposed to constant light, were given melatonin (10 μg subcutaneously), the incidence of estrus was reduced to 45 percent, while animals given diluent alone showed no change. This effect was observed for only the first day after injection; subsequently, animals that received melatonin returned to a normal estrous cycle. It was not possible to inhibit the onset of light-induced persistent estrus by means of daily injections of 10 μg of melatonin.

Thus, melatonin appears to satisfy the classical criteria for a hormone. (i) It is produced by a specialized glandular structure: only the pineal gland has the enzyme required for its synthesis (*7*, *10*). (ii) It is released into the circulation: melatonin is endogenously present in peripheral nerve, a tissue which does not make it but can take it up from the circulation. (iii) It has an effect on a distant target organ: it alters such gross factors as ovarian weight and the estrous cycle. (iv) It is not synthesized by the target organ; and hydroxyindole-*O*-methyl transferase could not be detected in rat or human ovary. (v) It is taken up by the target organ from the circulation. Melatonin is taken up by the brain, and concentrated by the pituitary and ovary; thus, its effects on ovarian weight and function could result from an action at any of these sites.

There is considerable evidence to show that the state of the pineal is related to environmental changes. Variations in day length produced changes in pineal cytology and serotonin content (*11*); exposure to constant light altered pineal size, cellular morphology, and chemical content. It is possible that the physiological disposition and actions of melatonin might be influenced by light (*12*).

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References and Notes

1. J. I. Kitay, *J. Clin. Endocrinol. Metab.* **14**, 622 (1954).
2. Y. Izawa, *Trans. Japan. Pathol. Soc.* **16**, 72 (1926); H. Simonnet, L. Thiéblot, T. Melik, *Ann. Endocrinol.* **12**, 202 (1951); J. I. Kitay, *Endocrinology* **54**, 114 (1954).
3. R. J. Wurtman, M. D. Altschule, U. Holmgren, *Am. J. Physiol.* **197**, 108 (1959); C. J. Meyer, R. J. Wurtman, M. D. Altschule, E. A. Lazo-Wasem, *Endocrinology* **68**, 795 (1961).

4. V. M. Fiske, *ibid.* **29**, 187 (1941); W. Jochle, *Endokrinologie* **33**, 287 (1956); J. D. Ifft, *Endocrinology* **71**, 181 (1962); R. J. Wurtman, W. Roth, M. D. Altschule, J. J. Wurtman, *Acta Endocrinol.* **36**, 617 (1961).
5. V. M. Fiske, J. Pound, J. Putnam, *Endocrinology* **71**, 130 (1962); W. B. Quay and A. Halevy, *Physiol. Zool.* **35**, 1 (1962); W. D. Roth, R. J. Wurtman, M. D. Altschule, *Endocrinology* **71**, 888 (1962).
6. A. B. Lerner, J. D. Case, R. V. Heinzelman, *J. Am. Chem. Soc.* **81**, 6084 (1959).
7. J. Axelrod, P. D. MacLean, R. W. Albers, H. Weissbach, in *Regional Neurochemistry*, S. S. Kety and J. Elkes, Eds. (Pergamon, New York, 1961), pp. 307–311.
8. L. Baschieri, F. DeLuca, L. Cramarossa, C. DeMartino, A. Oliverio, M. Negri, *Experientia* **19**, 15 (1963).
9. Melatonin-acetyl-H^3 prepared by the method of I. J. Kopin, C. M. B. Pare, J. Axelrod, H. Weissbach, *J. Biol. Chem.* **236**, 3072 (1961).
10. Hydroxyindole-*O*-methyl transferase was examined in 11 human pineal glands obtained at autopsy by the method of Axelrod and Weissbach [*J. Biol. Chem.* **236**, 211 (1961)]. The enzyme activity was sufficient to synthesize 240 ± 31 μg of melatonin per gram of tissue per hour. Calcified glands from aged subjects have as much enzyme activity as uncalcified glands.
11. W. B. Quay, *J. Morphol.* **98**, 471 (1956); personal communication.
12. We thank L. Stroud for technical assistance.

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