

Melatonin Synthesis in the Pineal Gland: Control by Light

Abstract. *In rats placed in continuous darkness for 6 days, there is a striking increase in the activity of melatonin-synthesizing-enzyme (hydroxyindole-O-methyl transferase) in the pineal gland, but no change in the activity of monoamine oxidase. Since melatonin appears to have a hormonal role in mammals, and its synthesis is confined to the pineal gland, the inhibition of hydroxyindole-O-methyl transferase by light may constitute a mechanism of neuroendocrine regulation.*

Melatonin (5-methoxy-N-acetyltryptamine) is produced in the mammalian pineal gland by the O-methylation of N-acetylserotonin (1, 2). Although the O-methylating enzyme, hydroxyindole-O-methyl transferase (HIOMT), has been sought in a wide variety of mammalian (3) and bird (4) tissues, it has been found only in the pineal gland. N-Acetylserotonin is formed by the N-acetylation of serotonin (5-hydroxytryptamine); this process is catalyzed by an enzyme found in many tissues, including the pineal gland (2). Since the mammalian pineal gland contains very large stores of serotonin (5), it seems likely that, under certain conditions, the activity of HIOMT may be the rate-limiting factor controlling the synthesis of melatonin in vivo. Pineal serotonin is also, presumably, metabolized by monoamine oxidase (5).

The mass (6), morphology (7), and biochemical composition (8) of the mammalian pineal gland can be altered by varying the amount of light or

darkness to which the animal is exposed: constant exposure to light decreases the weight of the pineal gland in the rat; this effect does not require the presence or normal functioning of the pituitary gland, gonads, adrenals, or thyroid (9). Constant illumination also decreases the size of pinealocyte nucleoli and the level of cytoplasmic basophilia. This has been interpreted as indicating a decrease in pineal RNA synthesis (7). The reverse histologic picture is found when rats are placed in continuous darkness.

It has recently been demonstrated that the administration of minute amounts of melatonin subcutaneously over long periods inhibits ovarian growth and the incidence of estrus in young rats (10). Larger doses produce a decrease in the size of seminal vesicles in male rats (11), and inhibit the response of the thyroid to methylthiouracil (12). The natural occurrence of melatonin in a certain tissue, peripheral nerve (13), which cannot synthesize it (3), but can concentrate it from the circulation (10), has been taken as evidence that melatonin is a hormone normally present in the mammalian circulation (10). The physiological disposition of intravenously-administered tritiated melatonin is altered in the rat exposed to continuous light, while minute doses of this compound interfere with the persistent estrus induced by light (10). Since the actions and disposition of melatonin seem to be related to light exposure in the mammal, studies were undertaken to determine whether the capacity of the rat pineal gland to synthesize melatonin from its immediate precursor was also subject to photic regulation. Our ex-

periments show that exposure of rats to constant darkness for as little as 6 days induces a striking increase in the activity of HIOMT in the pineal gland.

Sprague-Dawley female rats were placed in constant light (7), or constant darkness, except for a 25-watt red light bulb which was used while the cages were cleaned, in special rooms equipped with double-door light baffles and air conditioning. Light or darkness did not affect body weight, nor did they influence the length of gestation or litter size among newly-conceived pregnant rats. Vaginal smears were checked daily for about a week prior to enzyme assay in all groups, to confirm the presence of light-induced constant estrus. Animals were killed while still in their dark or light environment. Pineals were quickly dissected, weighed, and homogenized in 1.0 ml of 0.5M phosphate buffer, pH 7.9. Two or three pineal glands were pooled for each assay. A 0.6 ml sample was assayed for HIOMT activity, and a 0.1 ml sample was used for assay of monoamine oxidase activity, by methods previously described (3, 14).

When immature, 21-day-old rats were kept in constant darkness for 50 days, they showed a highly significant increase in HIOMT activity in the pineal gland (Table 1); pineal glands of rats maintained in constant light had one-half the HIOMT activity of animals exposed to diurnal lighting, and only one-tenth that of littermates exposed to constant darkness. Constant darkness was associated with a significant increase in the weight of the pineal gland, when compared with glands of rats kept in constant light; pineal glands of animals exposed to diurnal lighting were intermediate in weight. When HIOMT activity was expressed per unit weight of gland, rats exposed to constant darkness had more than six times the activity of light-treated littermates. Neither treatment produced any change in the activity of monoamine oxidase in the pineal gland, whether the activity was expressed per gland or per unit weight. Animals kept in continuous light for 50 days showed an increased incidence of estrous phases (proestrus, estrus, or metaestrus), while those in darkness were largely in diestrus.

An experiment was performed to determine whether lighting conditions could produce a difference in HIOMT activity in the pineal gland of mature rats, and within a relatively short pe-

Table 1. The effect of illumination on the activity of hydroxyindole-O-methyl transferase (HIOMT) and monoamine oxidase (MAO) in the pineal glands of female rats. The activity of HIOMT is expressed as μ moles of C^{14} -labeled melatonin synthesized per hour; MAO activity is expressed as μ moles of C^{14} -labeled indoleacetic acid formed per hour.

Treatment	Duration of treatment	Weight of pineal gland (mg)	Activity			Estrus (%)
			HIOMT		MAO (per mg)	
			Per gland	Per mg		
<i>Groups of 10 rats, each rat aged 21 days at onset of treatment</i>						
Dark	50 days	1.51 ± 0.08	30.1 ± 5.14	20.1 ± 3.03	1410 ± 218	23
Diurnal lighting	50 days	1.13 ± 0.06*†	5.5 ± 1.39*	4.9 ± 1.21*	1140 ± 175	50
Light	50 days	0.95 ± 0.05*	3.0 ± 0.74*	3.1 ± 1.02*	1460 ± 161	78
<i>Groups of 10 rats, each rat aged 78 days at onset of treatment</i>						
Dark	6 days	1.28 ± 0.03	22.8 ± 6.11	17.6 ± 4.57	1370 ± 20	50
Light	6 days	0.93 ± 0.02*	4.1 ± 2.62*	3.9 ± 1.55*	1640 ± 300	60
<i>Groups of 6 rats, treated from birth</i>						
Dark	55 days	1.26 ± 0.02	13.1 ± 0.64	10.3 ± 0.60	1160 ± 80	29
Light	55 days	0.86 ± 0.09*	4.1 ± 1.66*	4.8 ± 2.09*	1010 ± 120	67

* Differs from dark-treated, $p < 0.001$. † Differs from light-treated, $p < 0.05$.

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riod. When 78-day-old animals were exposed to constant darkness for 6 days, there was a fivefold increase in the melatonin-forming enzyme activity (Table 1) compared with animals kept in continuous light. Pineal glands of rats kept in constant darkness for 6 days were appreciably heavier than those of littermates maintained in light. Exposure to light for 6 days induced no significant change in the estrous cycle, or in the activity of monoamine oxidase in the pineal gland.

Another experiment was designed to determine whether exposure to constant light or darkness from birth produced correspondingly greater changes in the activity of HIOMT in the pineal gland, compared with the changes observed in the short-term experiments. The effect of darkness upon HIOMT activity was no greater in animals exposed for 55 days, from birth, than in adult rats treated for 6 days (Table 1). Again, exposure to constant light was associated with decreased weight of the pineal gland, unchanged monoamine oxidase activity, and an increase in the incidence of estrus.

About one third of the animals kept in constant light had no detectable HIOMT activity in the pineal gland. Male rats and female guinea pigs, which were not in constant estrus, also responded to constant light exposure with decreased HIOMT activity and unchanged monoamine oxidase activity.

That exposure to light can inhibit selectively the activity of the enzyme required for the synthesis of the pineal hormone, melatonin, is of especial interest in view of the possibility that this enzyme limits the rate of melatonin synthesis *in vivo*. Since melatonin appears to be released from the pineal gland into the circulation, and to act on distant organs as a hormone (10), the relationship presented here between illumination and HIOMT activity in the pineal gland may describe a new kind of neuroendocrine regulatory mechanism: control by light of the availability of a hormone by regulating the activity of an enzyme required for its synthesis. It is suggested that one means by which light exposure may induce an increase in ovary growth and in the incidence of estrus (15) is by inhibiting the synthesis of melatonin, a hormone which inhibits both these functions (10).

Information relating to environmental lighting could be transmitted to the pineal gland by several routes: (i) reti-

nal stimulation could be transmitted to the pineal gland through nerve pathways; (ii) light could act indirectly by influencing other circulating hormones, which in turn would affect enzyme activity; or (iii) light could impinge directly upon the mammalian pineal gland.

Previous work, in which light exposure was found to be related to the RNA content of the pineal gland (7), by histochemical estimations, suggests that the differences in enzyme activity described here may actually represent differences in the net rate of synthesis of enzyme protein. The fact that HIOMT activity in the pineal gland can be increased in the absence of light in adult animals, treated for a short period, as effectively as in immature animals treated from birth, suggests that cyclic variations in the activity of this enzyme may occur physiologically, perhaps even diurnally. Since light exposure and the incidence of estrus both show periodicity, it is possible that diurnal variations in light may influence the estrous cycle by influencing the synthesis of melatonin.

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