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Nocturnal Increase in the Sensitivity of the Syrian Hamster Pineal Gland to Isoproterenol Is Darkness Dependent[1] (42538)

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Abstract. The sensitivity of the Syrian hamster pineal gland to stimulation by isoproterenol is greatly increased in the latter half of the daily dark phase. This increased sensitivity requires a period of dark exposure for up to 6.5 hr. Also, if dark-maintained hamsters are exposed to light in the latter half of the night pineal melatonin levels drop precipitously but can be restimulated by isoproterenol administration. As the interval of light exposure continues, however, the pineal sensitivity to isoproterenol decreases.

In the rat, the administration of either norepinephrine (NE) (especially when preceded by the injection of a NE uptake inhibitor) or isoproterenol, a β-receptor agonist, during the photophase of the light:dark (LD) cycle readily stimulates production of melatonin within the pineal gland (1, 2). On the other hand, the daytime administration of these compounds to the Syrian hamster has no stimulatory action on pineal melatonin (3–5). It was recently discovered, however, that during the dark phase of the LD cycle the Syrian hamster pineal will respond to the catecholamine or its agonist. This was proven using two experimental paradigms. First, if isoproterenol is given prior to acute light exposure at night the normal light-associated reduction in pineal melatonin levels is delayed (6). Second, if either NE (preceded by an uptake inhibitor) or isoproterenol is injected into Syrian hamsters with light-suppressed melatonin levels at night, melatonin production within the pineal gland is increased (7–9). In both of these experimental paradigms the hamsters were exposed to 6.5–8 hr of darkness before a positive response to either NE or its agonists could be evoked.

The purpose of the first study in this series was to determine whether this period of dark exposure was necessary for the hamster pineal gland to subsequently respond to isoproterenol. In the second study we determined whether the interval after acute light exposure at night influenced the response of the hamster pineal gland to isoproterenol.

Materials and Methods. The animals used in these studies were adult male Syrian hamsters (80–100 g) purchased from Sasco (Omaha, NE). They were housed in groups of four to five per clear polycarbonate cage and food and water were available at all times. During a 1-week period of acclimation the animals were kept under a LD cycle of 14:10 with lights out at 2000 hr daily.

Experiment 1. Sixty-four hamsters (8 groups of 8 each) were used in this study. On the night of the experiment, the pineal glands were collected from 8 animals at the end of the LD cycle (at 2000 hr). Thereafter, 32 animals entered their normal dark phase while the remaining 24 hamsters were exposed to light (roughly 50 $\mu W/cm^2$ provided by cool white fluorescent bulbs) for the remainder of the experiment. At 0230 hr (after 6.5 hr of darkness) the pineal glands were collected from an additional 8 animals that had been exposed to darkness. Immediately following this, the remaining 24 dark-maintained hamsters were acutely exposed to light (roughly 50 $\mu W/cm^2$). This light intensity rapidly suppresses hamster pineal melatonin content (10). Thirty minutes after light onset (at 0300 hr) when pineal melatonin levels had reached daytime values, the pineal glands were collected from 8 hamsters and the remaining 16 animals were given a subcutaneous injection of 1 mg/kg (−)-iso-

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proterenol (Sigma Chemical Co.) in 0.1 ml saline. These animals were killed either 90 or 120 min later. When pineal glands were collected they were frozen on solid CO_2 and stored at −80°C for later melatonin analyses.

The hamsters that were maintained in continual light on the night of experiment were treated similarly. At 0300 hr the pineal glands were collected from 8 animals while the remaining 16 hamsters received a subcutaneous isoproterenol injection; these were killed 90 or 120 min later and their pineal glands were frozen and stored as above.

Experiment 2. Eighty hamsters (10 groups of 8 each) were used in this study. On the night of the experiment all animals entered darkness at the usual time (2000 hr). At 0230 hr (6.5 hr into the dark phase) the pineal glands of 8 hamsters were collected and frozen as above. The remaining 72 animals were acutely exposed to light (as above) to depress their high nocturnal melatonin levels. Twenty minutes after light onset the pineals were collected from 1 group and 16 other animals received an injection of isoproterenol; 40 min after light onset the pineals were collected from another control group and 16 additional animals received isoproterenol; finally, 80 min after light onset the pineals were collected from a third group of control animals and 16 other animals received isoproterenol. All isoproterenol-injected hamsters were killed at either 90 or 120 min after drug administration.

Pineal glands were analyzed for their melatonin content using a radioimmunoassay developed by Rollag and Niswender (11) and currently in use in this laboratory. Data were statistically analyzed using an ANOVA followed by a Student–Newman–Keuls multiple-range test.

Results. *Experiment 1.* At 2000 hr (end of the light phase) the mean melatonin concentration in the pineal gland was 128 ± 8 pg/gland (Fig. 1). In the animals that were then placed in darkness, pineal melatonin values rose to 449 ± 38 pg/gland at 0230 hr, 6.5 hr into the dark phase. The acute exposure of the dark-exposed hamsters to light caused a rapid decline in pineal melatonin concentrations such that 30 min after light onset (at 0300 hr) melatonin levels had again reached basal daytime levels (Fig. 1, solid points). When these light-suppressed animals were then treated with 1 mg/kg isoproterenol, pineal melatonin values rose markedly ($P < 0.001$) to high levels 90 and 120 min after drug administration.

Those animals exposed to continual light at night exhibited a markedly different response (Fig. 1, hollow points). At 0300 hr, after lights had been on all night, pineal melatonin levels were expectedly low; when these animals were subsequently given isoproterenol pineal melatonin values, measured 90 or 120 min later, were not elevated over those of the untreated control hamsters.

Experiment 2. At 6.5 hr into the dark phase the mean melatonin content of the pineal glands was 398 ± 30 pg/gland (Fig. 2). When these animals were then acutely exposed to light pineal melatonin values plummeted as in the first study. Thereafter, groups of hamsters were treated with isoproterenol at 20, 40, or 80 min after light onset. In each case drug administration was followed by significant ($P < 0.001$) rises in pineal melatonin levels 90 and 120 min later. As drug treatment was delayed after light onset, the melatonin response of the pineal gland to isoproterenol was also somewhat diminished.

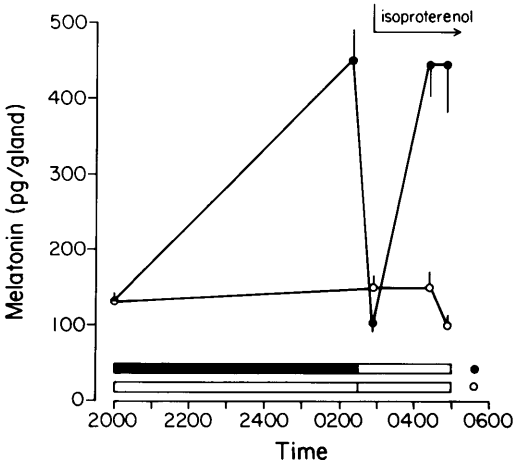

FIG. 1. Pineal response to isoproterenol in hamsters exposed to 6.5 hr of darkness (●) or constant light (○) before the administration of the drug. In the pineal of hamsters exposed to darkness, melatonin production was markedly stimulated following drug administration. In those animals exposed to light at night, isoproterenol could not promote melatonin production. The horizontal bars paralleling the abscissa indicate the LD cycles for the two groups of hamsters.

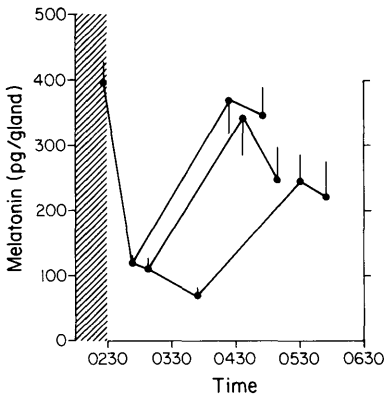

FIG. 2. Pineal response to isoproterenol in hamsters acutely exposed to light after 6.5 hr of darkness. The drug was given 20, 40, or 80 min after light onset. With progressive light exposure the magnitude of the melatonin response to isoproterenol gradually decreased.

Discussion. In both experiments 1 and 2 we confirmed that the pineal glands of Syrian hamsters produced melatonin in response to isoproterenol administration when the drug was given in the latter half of the dark phase. This is consistent with previous observations that only during this interval is isoproterenol (or NE) capable of promoting melatonin production in the pineal gland of the Syrian hamster (7–9). Furthermore, these studies show that dark exposure at night is a necessary aspect of the 24-hr rhythm in the sensitivity of the hamster pineal to isoproterenol and, also, as the interval after acute light onset at night is prolonged the ability of isoproterenol to stimulate melatonin production decreases.

Clearly, when hamsters were not given 6.5 hr of darkness at night prior to isoproterenol injection the pineal gland merely maintained low daytime melatonin values following the administration of the drug (Fig. 1). The mechanisms by which darkness sensitized the pinealocytes to the β-agonist cannot be determined from this study. Light at night interrupts the neural signal between the central nervous system and the pineal gland which causes the release of neurotransmitters from the sympathetic nerve terminals within the gland (12). Perhaps light exposure during the normal dark period suppressed the release of some factor which normally up-regulates the postsynaptic receptors on the pinealocyte membranes. A variety of neurotransmitters has been identified in the mammalian pineal gland but the functions of these compounds remain unknown (2, 13). It is interesting that in the Syrian hamster pineal melatonin concentrations do not normally rise until the latter half of the dark phase (14, 15). This lag in the melatonin rise is possibly due to a necessary darkness-associated up-regulation of the β-receptors on the pinealocyte membrane during the first 4–5 hr of dark exposure. If this occurs, however, it is not a universal feature in mammals since in many species darkness onset is accompanied by an almost immediate increase in pineal melatonin production (16).

The hamster pineal response to isoproterenol seems to become progressively less robust when the duration of acute light exposure is increased (Fig. 2). Thus, the most marked melatonin increase occurred when the animals received the isoproterenol 20 min after light exposure at night; when the drug was given 80 min after light onset the melatonin response was depressed although it certainly was not absent. The findings suggest that if the isoproterenol injection had been further delayed, eventually the pineal gland would not have responded with increased melatonin production. The loss of sensitivity of the pinealocyte to the β-agonist could be due to one of the following factors. Perhaps the sensitivity of the β-receptors to the drug has a finite limit of only one to several hours. Under LD conditions of 14:10 the hamster pineal melatonin peak is of short duration (1 or 2 hr) (14, 15) suggesting that the above-mentioned explanation could have some validity. Another explanation may relate the fact that when the lights were turned on, the factor which normally up-regulates the β-receptors was no longer being released and, as a result, the sensitivity of the β-receptors gradually waned.

In summary, the increased sensitivity of the Syrian hamster pineal gland to isoproterenol at night depends on the animals' exposure to darkness for an interval up to 6.5 hr. Continual light exposure at night not only prevents the development of sensitivity to the drug but, after hamsters are acutely exposed to light at night, the susceptibility of pineal melatonin synthesis to stimulation by isoproterenol diminishes with increased duration of light exposure.

1. Zatz M. Pharmacology of the rat pineal gland. In: Reiter RJ, Ed. The Pineal Gland. Vol. I. Anatomy and Biochemistry. Boca Raton, FL, CRC, pp229–242, 1981.
2. Ebadi M. Regulation of the synthesis of melatonin and its significance to neuroendocrinology. In: Reiter RJ, Ed. The Pineal Gland. New York, Raven Press, pp1–37, 1984.
3. Binkley S. Comparative biochemistry of the pineal gland of birds and mammals. Amer Zool **16**:57–70, 1976.
4. Lipton JS, Petterborg LJ, Steinlechner S, Reiter RJ. *In vivo* responses of the pineal gland of the Syrian hamster to isoproterenol or norepinephrine. In: Reiter RJ, Ed. The Pineal and Its Hormones. New York, Alan R. Liss, pp107–116, 1982.
5. Steinlechner S, King TS, Champney TH, Spanel-Borowski K, Reiter RJ. Comparison of the effects of β-adrenergic agents on pineal serotonin *N*-acetyltransferase activity and melatonin content in two species of hamsters. J Pineal Res **1**:23–30, 1984.
6. Steinlechner S, King TS, Champney TH, Richardson BA, Reiter RJ. Pharmacological studies on the regulation of *N*-acetyltransferase activity and melatonin content of the pineal gland of the Syrian hamster. J Pineal Res **2**:109–120, 1985.
7. Vaughan GM, Lasko J, Coggins SH, Pruitt BA Jr, Mason AD Jr. Rhythmic melatonin response of the Syrian hamster pineal gland to norepinephrine *in vitro* and *in vivo*. J Pineal Res **3**:235–250, 1986.
8. Reiter RJ, Oaknin S, Troiani ME, Li K. Factors controlling melatonin rhythmicity in the pineal gland of the Syrian hamster. Annu Rev Chronopharmacol **3**: 41–44, 1986.
9. Reiter RJ, Vaughan GM, Oaknin S, Troiani ME, Cozzi B, Li K. Norepinephrine or isoproterenol stimulation of pineal *N*-acetyltransferase activity and melatonin content in the Syrian hamster is restricted to the second half of the daily dark phase. Neuroendocrinology **45**:249–259, 1987.
10. Brainard GC Jr, Richardson BA, King TS, Matthews SA, Reiter RJ. The suppression of pineal melatonin content and *N*-acetyltransferase activity by different light irradiances in the Syrian hamster. Endocrinology **113**:293–296, 1983.
11. Rollag MD, Niswender GD. Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimes. Endocrinology **98**:482–489, 1976.
12. Moore RY. Central neural control of circadian rhythms. In: Ganong WF, Martini L, Eds. Frontiers in Neuroendocrinology. New York, Raven Press, pp185–206, 1978.
13. Ebadi M, Chan A, Hammad H, Govitrapong P, Swanson S. Serotonin *N*-acetyltransferase and its regulation by pineal substances. In: Reiter RJ, Ed. The Pineal and Its Hormones. New York, Liss, pp21–34, 1982.
14. Panke ES, Reiter RJ, Rollag MD, Panke TW. Pineal serotonin *N*-acetyltransferase activity and melatonin concentrations in prepubertal and adult Syrian hamsters exposed to short daily photoperiods. Endocrinol Res Commun **5**:311–324, 1978.
15. Panke ES, Rollag MD, Reiter RJ. Pineal melatonin concentrations in the Syrian hamster. Endocrinology **104**:194–197, 1979.
16. Reiter RJ. The role of light and age in determining melatonin production in the pineal gland. In: Axelrod J, Fraschini F, Velo GP, Eds. The Pineal and Its Endocrine Role. New York, Plenum, pp227–241, 1983.

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