Journal of Cellular Biochemistry 44:55–60 (1990)

mRNA Transcription Determines the Lag Period for the Induction of Pineal Melatonin Synthesis in the Syrian Hamster Pineal Gland

Aldo Gonzalez-Brito, Maureen E. Troiani, Armando Menendez-Pelaez, Maria J. Delgado, and Russel J. Reiter

Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7762

The nocturnal pattern of Syrian hamster pineal melatonin synthesis is characterized by a 6–8 h lag period, followed by a late-night, short-duration peak in both N-acetyltransferase (NAT) activity and melatonin content. Administration of cycloheximide (20 mg/kg body weight) given either at the time of lights out or 4 h into the dark phase to Syrian hamsters blocked the nocturnal increase in both pineal NAT activity and melatonin content. Actinomycin D (5 mg/kg body weight) prevented the nocturnal increase in both constituents only when it was administered at darkness onset, being significantly less effective when injected after 4 h of dark exposure. Reinduction of melatonin production by isoproterenol (2 mg/kg body weight) administration to acutely light-exposed animals during late darkness was prevented by cycloheximide, but not by actinomycin D administration. The results suggest that whereas Syrian hamster pineal melatonin production requires protein synthesis both early and late in the dark phase, the transcription of a putative NAT-related mRNA, which occurs only during the early night, seems to determine the lag period in melatonin synthesis and pineal responsiveness to β-adrenergic receptor agonist stimulation.

Key words: actinomycin D, cycloheximide, nocturnal melatonin production, isoproterenol, β-adrenergic receptors

In the Syrian hamster [1–4], as in all mammalian species studied [5–7], the synthesis of melatonin is controlled by norepinephrine (NE) released from sympathetic nerve endings whose cell bodies are in the superior cervical ganglia. The nocturnal pattern of pineal melatonin synthesis in Syrian hamsters exposed to a 14:10 h light:dark (LD) cycle is characterized by a 6–8 h lag period followed by a late-night, short-duration peak in both N-acetyltransferase (NAT) activity and melatonin content [1–4]. Whereas the administration of a β-adrenergic receptor agonist to the albino rat induces an increase in melatonin synthesis at any time during the LD cycle [7], the

Received February 8, 1990; accepted May 16, 1990.

responsiveness of the hamster pineal gland to β-receptor agonist stimulation seems restricted, both in vivo and in vitro, to the latter half of the daily dark phase [4,8–11]. This responsiveness requires that hamsters be exposed to darkness for a period up to 6.5 h before β-receptor stimulation induces melatonin synthesis [12]. A similar lag period before the induction of melatonin production is required when hamster pineal glands are incubated with the adenylate cyclase activator forskolin [13]. These findings suggest that intracellular mechanisms seem to determine the rapidity with which the hamster pineal responds to β-agonist stimulation.

Recently, we reported [11] that the in vivo administration of isoproterenol (ISO), a β-adrenergic receptor agonist, for 4 h before the onset of darkness induced a 4 h advance in the onset of the nighttime increase in both NAT activity and melatonin levels in the hamster pineal. Moreover, ISO (1 mg/kg) administered every 2 h to animals kept in light during the night, induced an increase in melatonin synthesis after 4–6 h [14]. The results indicate that the Syrian hamster pineal gland can respond in vivo to continuous β-adrenergic stimulation, but a lag period of 4–6 h is required before there is an increase in melatonin synthesis. The present experiments were conducted in an attempt to clarify the intracellular mechanisms that account for the relatively long lag period in Syrian hamster pineal melatonin production.

MATERIALS AND METHODS

Adult male Syrian hamsters (*Mesocricetus auratus*), averaging 140 g body weight (BW), were housed in groups of six to eight animals per cage, with food and water provided ad libitum and exposure to LD cycles (14–10 h) for a minimum of 1 week before being used in a particular study. Lights were out daily from 2000 to 0600 h, unless otherwise noted. Animals were killed by decapitation and the pineals were removed, frozen on solid CO_2, and stored at − 60°C until assayed for NAT activity and melatonin. The glands were individually assayed for NAT using the procedure outlined by Champney et al. [15]; melatonin levels were estimated using a radioimmunoassay outlined by Rollag and Niswender [16]. Data, expressed as means ± SEM, were statistically analyzed using an analysis of variance (ANOVA) followed by a two-tailed Student *t*-test.

In the first study, six groups (8–10 animals each) of Syrian hamsters were used. One group of animals was killed by decapitation at the end of the light phase (2000 h), and their pineal glands were collected to determine daytime levels of pineal NAT activity and melatonin. The remaining five groups of hamsters entered darkness at the usual time (2000 h). Two of these groups received, at 2000 h, a single injection of either the protein synthesis inhibitor cycloheximide (20 mg/kg BW in a solution of 20% ethanol/80% saline) or the RNA synthesis inhibitor actinomycin D (5 mg/kg BW in a solution of 30% ethanol/80% saline), respectively. The volume of fluid administered during the injections was 0.1 cc. Two additional groups received injections of the same drugs at 2400 h, 4 h after darkness onset. A control group of hamsters received an intraperitoneal (i.p.) injection of the alcoholic saline solution at both 2000 and 2400 h. Because either cycloheximide or actinomycin D could possibly act presynaptically on the postganglionic sympathetic neurons in the pineal to prevent the endogenous release of NE and thereby the nighttime pineal rise in melatonin production, all five groups of hamsters were given, at two hourly intervals, subcutaneous injections of the β-receptor agonist ISO (0.5 mg/kg in saline) between 2000 and 0200 h; thus, each animal received four injections of ISO. All animals were decapitated at 0400 h, and

their pineal glands were frozen on solid CO_2. All procedures carried out at night were done with the animals exposed to a dim red light that was insufficient to influence pineal melatonin production [17].

In a second experiment, five groups (8–9 hamsters each) of animals entered the normal dark period at 2000 h; 7.5 h later, at 0330 h, all animals were acutely exposed to white light (cool white fluorescent light that had an irradiance of roughly 100 $\mu W/cm^2$ to suppress pineal melatonin production [17]). Thirty min later (at 0400 h), four groups of hamsters were injected with either vehicle, ISO (2 mg/kg subcutaneously in saline), ISO plus cycloheximide (20 mg/kg BW i.p.), or ISO plus actinomycin D (5 mg/kg BW i.p.), respectively. The fifth group of animals was killed at 0400 h to prove that acute light exposure for 30 min had indeed suppressed pineal NAT activity and melatonin content. The four groups of animals that received injections at 0400 h were subsequently killed at 0600 h, and their pineal glands were collected.

(−)(−)Isoproterenol, cycloheximide, and actinomycin D were purchased by Sigma Chemical Co. (St. Louis, MO). Other chemicals, all of reagent grade, were obtained from commercial sources.

RESULTS

Experiment 1

ISO administration at night caused a highly significant rise in pineal NAT activity (Fig. 1, left, $P < 0.001$ controls at 0400 h vs. controls at 2000 h). The protein synthesis inhibitor cycloheximide, given at either 2000 or 2400 h, significantly restrained the rise in the activity of pineal NAT. By contrast, the mRNA synthesis inhibitor actinomycin D was effective in curtailing the rise in NAT activity only when the drug was given at 2000 h.

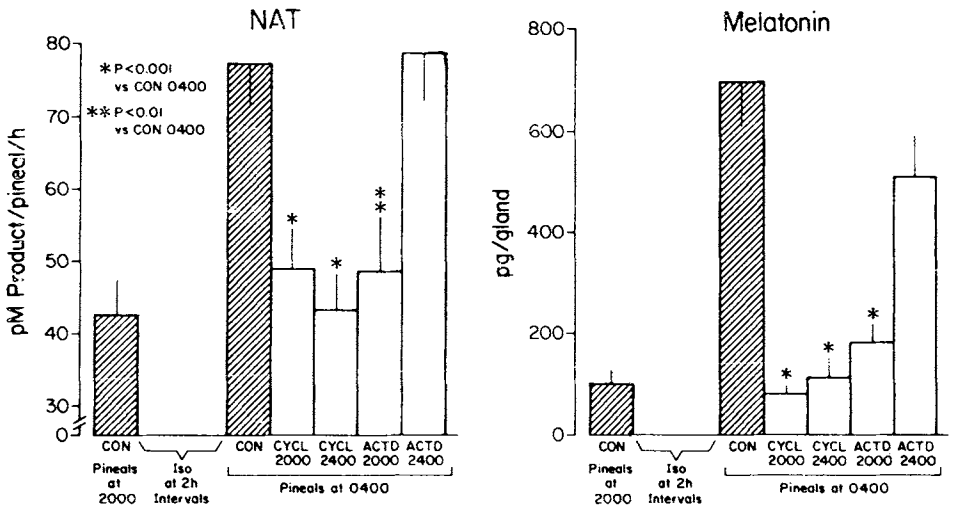

Fig. 1. NAT activity and melatonin content of Syrian hamster pineal glands collected at the end of the light phase (2000 h) or late in the dark phase (0400 h). Animals were treated with isoproterenol (ISO) at 2 h intervals during darkness. Some hamsters received either cycloheximide (CYCL) or actinomycin D (ACTD) at either lights out (2000 h) or at 4 h into the dark phase (2400 h). CON, control hamsters. Data are means ± SEM.

As with NAT activity, pineal melatonin levels rose markedly at night in hamsters treated with ISO at 2 h intervals (Fig. 1, right, $P < 0.001$ controls at 0400 h vs. controls at 2000 h). Again, cycloheximide effectively suppressed the rise in pineal melatonin content whether it was given at 2000 or 2400 h, whereas actinomycin D, only when given at 2000 h, was able to limit pineal melatonin production.

Experiment 2

Hamsters exposed to 30 min of light at night had low pineal NAT activity at 0400 h (Fig. 2, left); following ISO administration, NAT activity increased significantly 2 h later at 0600 h. The ISO-induced rise in NAT activity was inhibited by cycloheximide administration but was unaffected by the mRNA synthesis inhibitor actinomycin D.

Melatonin levels responded similarly to the treatments (Fig. 2, left). Light-exposed animals killed at 0400 h had low pineal melatonin values. These increased markedly after ISO administration, with the rise being suppressed by the protein synthesis inhibitor cycloheximide, but not by actinomycin D.

DISCUSSION

In the Syrian hamster [18], as in the rat [19,20], attainment of the nocturnal peaks of pineal NAT activity and melatonin content requires protein synthesis, as indicated by the fact that cycloheximide administration prevents or greatly reduces the nighttime rises in these constituents (Fig. 1). The transcription of mRNA for NAT also

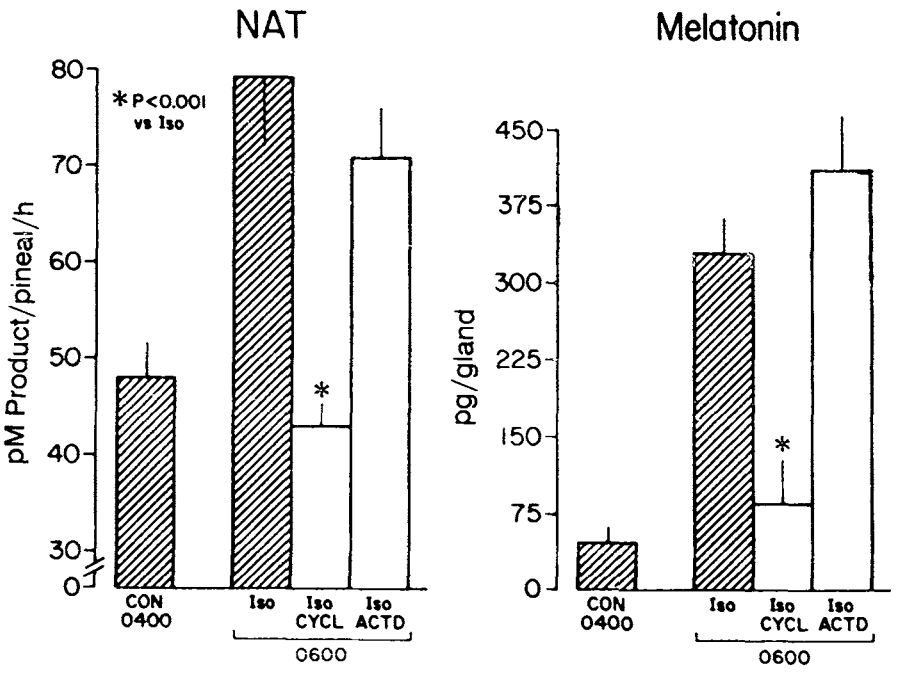

Fig. 2. NAT activity and melatonin content of Syrian hamster pineal glands collected at either 0400 h or at 0600 h. Animals killed at 0600 h had received isoproterenol (ISO) alone or in combination with cycloheximide (CYCL) or actinomycin D (ACTD) at 0400 h. CON, control hamsters. Data means ± SEM.

seems to be required. The inhibition of mRNA synthesis by actinomycin D administration at 2000 h largely blocked the nocturnal increases in both pineal NAT activity and melatonin content. However, when administered at 2400 h, the drug was without effect on the subsequent rise in pineal melatonin (Fig. 1); these findings suggest that the 4 h interval immediately following lights out is associated with mRNA transcription, which is required for maximal NAT activity and melatonin production when they are measured at 0400 h, 8 h after darkness onset. These parameters of Syrian hamster pineal cell biology are summarized in Figure 3.

Once nocturnal melatonin synthesis begins, exposure of Syrian hamsters to light is accompanied by an abrupt decrease in both pineal NAT activity and melatonin content [21–23]. ISO administration (2 mg/kg) to acutely light-exposed animals after 6.5 h of dark exposure induces, within 2 h, a significant increase in both constituents (Fig. 2). The restimulation of melatonin production after light-induced suppression was largely blocked by cycloheximide, suggesting that, in addition to posttranslational mechanisms, the restimulation of depressed NAT activity and melatonin also requires protein synthesis. However, transcription of additional new mRNA seems unnecessary, since the administration of actinomycin D was without influence on the subsequent rises in NAT activity and melatonin levels induced by ISO.

In the albino rat, the lag period for the induction of NAT activity by beta-agonists varies with the length of exposure to light before darkness onset [19,20]. If rats have been exposed to light, and thereby reduced sympathetic neuronal activity to the pineal gland, for more than 12 h, the lag period is 1–2 h; for NAT activity to increase subsequently, both mRNA transcription and protein synthesis are required. Conversely, the re-induction by β-receptor agonists of light-suppressed NAT activity at night occurs almost immediately and does not require additional new mRNA transcription [19,20]. Compared to the rat, in the Syrian hamster, the nocturnal synthesis of pineal melatonin, after 14 h of light exposure, requires a much longer lag period. During the first 4 h of darkness, when no increase in melatonin production is detected, the synthesis of mRNA probably related to the de novo synthesis of NAT, delays the

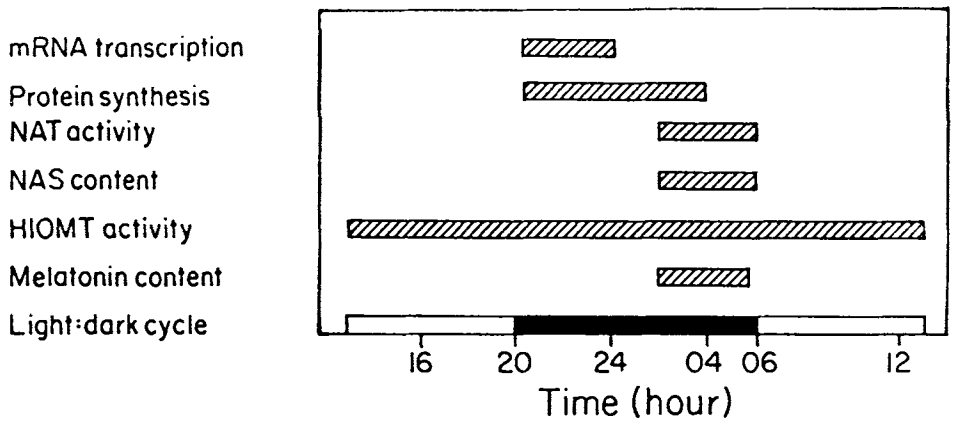

Fig. 3. Presumptive maximal levels (represented by the cross-hatched bars) of various constituents in the pineal gland of the Syrian hamster over a light:dark (LD 14:10) cycle. HIOMT, hydroxyindole-O-methyltransferase; NAS, N-acetylserotonin (the immediate precursor of melatonin); NAT, N-acetyltransferase.

attainment of both the nocturnal peaks of pineal NAT activity and melatonin content. Once the mRNA precursor is available, restimulation of NAT activity after light exposure during darkness is prevented only by cycloheximide, as in the rat [19,20]. The availability of mRNA after 6.5 h of dark exposure probably explains the observation that ISO can restimulate melatonin synthesis in the hamster pineal gland only during the latter half of the dark phase [4,8–11]. The fact that hamster pineals, collected during the daytime and incubated with the postreceptor stimulator of adenylate cyclase (forskolin) for a period longer than 4 h, exhibit an increase in melatonin production, which is blocked by actinomycin D, strongly support this interpretation of the present results (Santana et al., unpublished).

The molecular mechanisms that account for the slower rate of transcription of a putative mRNA precursor of pineal NAT in the Syrian hamster, compared to the rat, remains to be studied. The differential lag periods for mRNA transcription likely explain the different nocturnal patterns of melatonin production in the Syrian hamster and rat pineal gland [24].

ACKNOWLEDGMENTS

This work was supported by NSF Grant DCB 8711106; A.G.B. was supported by a fellowship from a collaboration agreement between Caja Canarias and the Autonomous Government of the Canary Islands.

REFERENCES

1. Rudeen PK, Reiter R, Vaughan MK: Neurosci Lett 1:225, 1975.
2. Panke ES, Rollag MD, Reiter RJ: Endocrinology 104:194, 1979.
3. Roberts AC, Martenz ND, Hastings MH, Herbert J: Endocrinology 117:141, 1985.
4. Reiter RJ, Oaknin S, Troiani M, Li K: In Reinberg A, Smolensky M, Labreque G (eds): "Annual Review of Chronopharmacology," Vol 3. Oxford: Pergamon Press, 1986, pp 41–44.
5. Illnerova H: Neuroendocrinology 16:202, 1974.
6. Binkley S, Klein DC, Weller JC: J Neurochem 26:61, 1976.
7. Zatz M: In Reiter RJ (ed): "The Pineal Gland, Anatomy and Biochemistry," Vol. 1. Boca Raton, FL: CRC, 1981, pp 229–242.
8. Vaughan GM, Lasko J, Coggins SH, Pruitt Jr BA, Mason Jr AD: J Pineal Res 3:235, 1986.
9. Reiter RJ, Vaughan GM, Oaknin S, Troiani ME, Cozzi B, Li K: Neuroendocrinology 45:249, 1987.
10. Vaughan GM, Reiter RJ: Endocrinology 120:1682, 1987.
11. Santana C, Guerrero JM, Reiter RJ, Puig-Domingo M, Gonzalez-Brito A: Neuroendocrinology 48:229, 1988.
12. Reiter RJ, Puig-Domingo M, Guerrero JM, Gonzalez-Brito A: Proc Soc Exp Biol Med 185:219, 1988.
13. Santana C, Guerrero JM, Reiter RJ, Gonzalez-Brito A, Menendez-Pelaez A: Biochem Biophys Res Commun 155:209, 1988.
14. Gonzalez-Brito A, Reiter RJ, Santana C, Menendez-Pelaez A, Guerrero JM: Brain Res 475:393, 1988.
15. Champney TH, Holtorf AP, Steger RW, Reiter RJ: J Neurosci Res 11:59, 1984.
16. Rollag MD, Niswender GD: Endocrinology 98:59, 1976.
17. Reiter RJ: Ann NY Acad Sci 453:215, 1985.
18. Steinlechner S, King TS, Champney TH, Richardson BA, Reiter RJ: J Pineal Res 2:109, 1985.
19. Romero JA, Zatz M, Axelrod J: Proc Natl Acad Sci USA 72:2107, 1975.
20. Romero JA: Fed Proc 35:1157, 1976.
21. Rollag MD, Panke ES, Trakulrungsi WK, Trakulrungsi C, Reiter RJ: Endocrinology 106:231, 1980.
22. Brainard GC, Richardson BA, King TS, Matthews SA, Reiter RJ: Endocrinology 113:293, 1983.
23. Reiter RJ, Puig-Domingo M, Guerrero JM, Gonzalez-Brito A: Proc Soc Exp Biol Med 185:219, 1987.
24. Reiter RJ: Life Sci 40:21, 1987.