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Rhythmic Melatonin Response of the Syrian Hamster Pineal Gland to Norepinephrine In Vitro and In Vivo

George M. Vaughan, James Lasko, Sandy H. Coggins, Basil A. Pruitt, Jr., and Arthur D. Mason, Jr.

US Army Institute of Surgical Research, Fort Sam Houston, Texas

Norepinephrine (NE, 10^{-6} M) stimulated melatonin accumulation in the incubation medium of rat (but not Syrian hamster) pineals taken at the end of the light phase. However, NE elevated melatonin accumulation in the medium of pineals taken after 20 min of light exposure of animals of either species at 6 h into the 10-h dark phase. A dose response to 10^{-7}–10^{-5} M NE was observed in both the medium and pineals upon incubation of pineals taken from rats at 4 h into the light phase and from hamsters after 20 min light exposure at 6 h into the dark phase. Approximately 95% of the melatonin present was in the medium. The incubation time was 4 h in all cases. Subcutaneous injection of 1 μg/g NE (either at the end of the light phase or after 30 min of light at 6 h into the dark phase) did not stimulate in vivo Syrian hamster pineal melatonin content determined 1 or 2 h after injection, whether the hamsters were placed in light or darkness after the injection. However, after 30 min of light beginning at 6 h into dark, injection of 5 μg/g desipramine (DMI, a blocker of catecholamine uptake into nerve endings) allowed a dramatic hamster pineal melatonin response to additional injection of 1 μg/g NE, observed at 1 and 2 h in light after injection. A small effect of DMI alone was seen. DMI also potentiated the effect of NE (each 10^{-6} M) on melatonin

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In conducting the research described in this report the investigators adhered to the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and in adherence with the Guide for the Care and Use of Laboratory Animals, NIH publication 80-23.

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Accumulation in the medium of incubated hamster pineals taken after a short light exposure at night. No significant stimulatory effect of NE and/ or DMI was seen in vivo or in vitro near the middle of the light phase. Measurement of melatonin in the incubation medium is a useful method for studying pineal function. The Syrian hamster pineal has rhythm of sensitivity to NE (sensitivity evident at night) and even at night is protected by neuronal uptake from circulating NE-induced stimulation of melatonin production. NE appears to be the neurotransmitter for stimulation of pineal melatonin production in the Syrian hamster. The sensitivity rhythm and uptake protection might provide specificity of control of the nightly melatonin signal by reducing the chance of a melatonin response during the day or a response to circulating catecholamines from general sympathetic stimuli.

Key words: hamster, rat, melatonin, sympathetic, rhythms, desipramine

INTRODUCTION

Pineal melatonin synthesis in the rat is stimulated by the adrenergic neurotransmitter norepinephrine (NE) acting predominantly through a beta-receptor mechanism [Klein, 1972; Brownstein et al., 1973; Vanecek et al., 1985]. Both beta-blockade and sympathetic neuronal lesions interrupt or prevent the normal nocturnal surge of pineal melatonin synthesis or of circulating or excreted melatonin in rats, Syrian hamsters, and humans [Eichler and Moore, 1971; Deguchi and Axelrod, 1972; Reiter and Sorrentino, 1972; Reiter, 1972; Kneisley et al., 1978; Klein and Moore, 1979; Panke et al., 1979; Tetsuo et al., 1981; Lipton et al., 1981; Cowan et al., 1983; Vaughan, 1984; Arendt et al., 1985; Vaughan and Reiter, 1985]. Taken together, these findings indicate similarity of these three species with regard to sympathetic control of melatonin production as revealed by neural lesions and beta-blockade. In the rat, injection of NE or isoproterenol [Deguchi and Axelrod, 1972; Axelrod, 1974] and the general sympathetic stimuli of insulin hypoglycemia [Lynch et al., 1973; Champney et al., 1985] or other conditions [Parfitt and Klein, 1976; Lynch et al., 1977; Allen et al., 1981; Seggie et al., 1985] increase daytime melatonin synthesis, an effect magnified by blocking catecholamine uptake in the nerve endings or by prolonged light exposure of the rats.

In contrast, inability to stimulate daytime pineal melatonin synthesis with norepinephrine or isoproterenol administration or insulin hypoglycemia in Syrian hamsters has recently led authors to express doubt about the role of NE as the pineal sympathetic neurotransmitter in this species [Lipton et al., 1982; Steinlechner et al., 1984, 1985; Champney et al., 1985]. Similar lack of rise in circulating human melatonin after beta-agonists [Moore et al., 1979; Krautgasser-Gasperotti et al., 1983; Vaughan, 1984], insulin hypoglycemia, and many other sympathetic stimuli [Gupta et al., 1983; Vaughan, 1984] has been noted and suggests that the Syrian hamster may provide a better model than the rat for sympathetic control of melatonin production in humans.

One purpose of the present study was to re-examine whether the putative pineal transmitter itself, NE, stimulates pineal melatonin production in the Syrian hamster. Experimental conditions were found for demonstrating this response. The findings appear to explain the previously reported ineffectiveness of adrenergic agents and strongly implicate NE as the pineal neurotransmitter for stimulation of melatonin production in the Syrian hamster. Another purpose was to determine whether measurement of melatonin in the medium of single pineal glands incubated without a radioactive precursor for a short time just after sacrifice of the animals can be used to investigate pineal function.

MATERIALS AND METHODS

Young adult male Syrian hamsters and Sprague-Dawley rats were maintained in our animal facility in a cycle of 14 h light and 10 h dark (lights off 2000–0600 h) for at least 3 wk prior to the experiments. Animals were decapitated by guillotine, alternating among the groups in an experiment, and the heads were placed upside down on wet ice. Pineals were then removed (in the order in which the heads were put on ice) and either incubated or frozen at −60°C for later melatonin analysis. For animals sacrificed in the dark (under two 25-W globes with red Kodak No. 1A filters), the heads on ice were brought into the light for further processing.

In experiments involving incubation, 0.9 to 1 ml Minimal Essential Medium (Flow Laboratories No. 11-100-22, Auto Pow, without glutamine, with phenol red and with our addition of $NaHCO_3$ 2 g/L, gentamycin 50 mg/L, and then fetal calf serum to 10% of the total volume, final pH 7.4) was added to 19-×-40-mm glass vials for incubation. The manufacturer indicates that L-tryptophan is 10 mg/L, and we measured the following concentrations: Na^+ 142, K^+ 5.4, Cl^- 128, and bicarbonate 22 mEq/L; Ca^{++} 7.2, P as PO_4 3.4, Mg^{++} 1.6, and glucose 100 mg/dl; and osmolality 280 mosm/kg. The vials with medium were pregassed in a Dubnoff shaker in a 95% O_2 and 5% CO_2 atmosphere at 37°C bath temperature for 1 h. With alternation among groups, single pineals were added to the vials followed by addition of nothing or a drug (previously dissolved in the same medium) in a 10- or 100-μl aliquot for a total incubation volume of 1 ml. Then each pineal was incubated for 4 h under the conditions of the pregassing phase, and medium was frozen for later melatonin radioimmunoassay [RIA; Vaughan et al., 1985], using the Rollag antibody [Rollag and Niswender, 1976]. Some vials contained no pineal during incubation in order to test for crossreactivity of the contents. Melatonin was not detectable in such medium without or with the drugs used at their highest concentrations. L-norepinephrine bitartrate (A-9512) was purchased from Sigma Chemical Co. (St. Louis, MO), and desipramine HCl (MDL 9384A) was a gift from Dr. W.J. Hudak of Merrell Dow Research Institute (Cincinatti, OH). The least detectable melatonin content in a vial was 50 pg for a hamster and 500 pg for a rat pineal medium.

In one incubation experiment, pineals were retrieved at the end and frozen. In in vivo experiments, pineals were frozen simply after removal from the animals. Pineals were sonicated in 0.1 ml pH 7.0 phosphate-buffered saline with 0.1% gelatin, diluted to 1 ml with this buffer, and refrozen

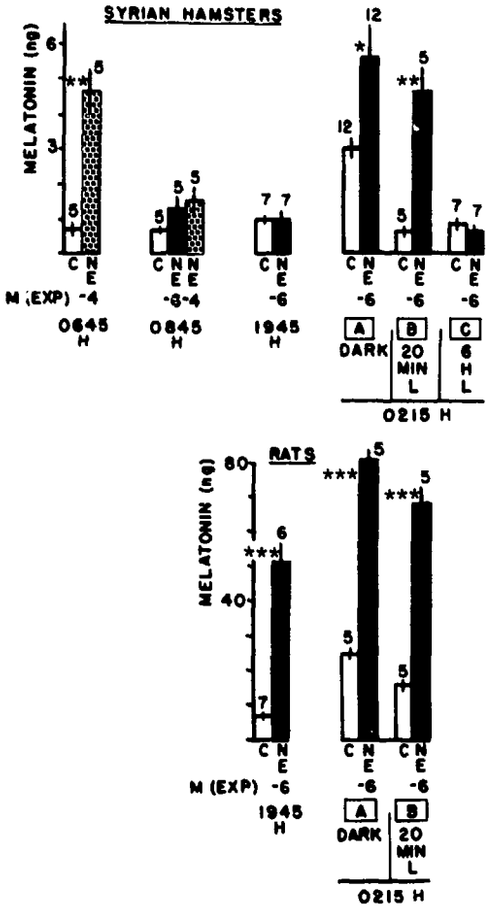

Fig. 1. Melatonin in the medium (mean ± SE). Pineals were taken from the animals at the times indicated (H) and incubated individually for 4 h (n/group indicated above the bars) without (C) or with (NE) norephinephrine. M (EXP): NE molarity as the exponent of 10; boxed symbols A,B,C: pineals taken from the animals at 0215 h, after the animals' exposure to 0, 20 min or 6 h of light (L) just prior to sacrifice; *, $P < 0.05$, **, $P < 0.01$ (NE vs C).

until assay. The least detectable melatonin was 10 pg/pineal. Samples from all groups for any statistical comparison were in one assay.

Statistical analysis was by t-test with Bonferroni correction for multiplicity of nonindependent comparisons, analysis of covariance, or two-way analysis of variance [Dixon, 1983].

Experiment (exp.) 1

The pineal from hamsters and rats was taken for incubation at the times indicated (Fig. 1). At 0215 h, animals were sacrificed either in the dark (A) or after exposure to light either for the preceding 20 min (B) or for the entire time that night prior to sacrifice (C). Condition C (extension of the previous light phase by approximately 6 h) was employed only in hamsters, as was sacrifice at 0645 h and 0845 h. Melatonin in the medium was determined.

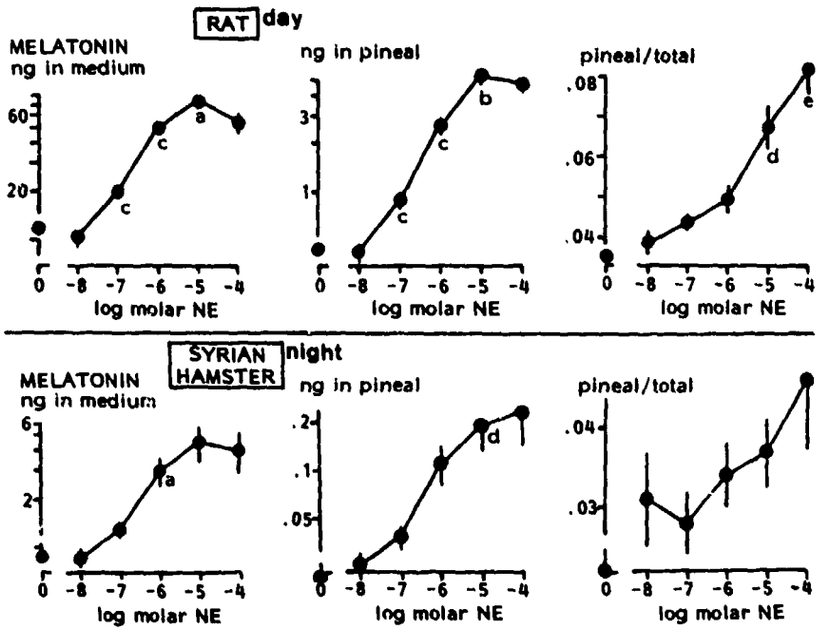

Fig. 2. NE dose-response relationships of melatonin (6/group, mean ± SE) in the medium or in the pineal after incubation. The abscissal 0 indicates no added NE. Pineals were taken from rats at 1000 h (4 h into the light phase) and from hamsters at 0215 h (approximately 6 h into the dark phase) after 20 min exposure of the hamsters to light just prior to sacrifice. Threshold *P* for comparison to the next lower NE dose group, a < 0.05, b < 0.01, c < 0.001; to the group at 10^{-8} M, d < 0.05; to the group at 10^{-6} M, e < 0.05.

Experiment 2

The pineal was taken for incubation from rats at 4 h into the light phase and from hamsters after 20 min of light exposure at 6 h into the dark phase, in order to investigate dose-response relationships with NE in the medium at various concentrations (Fig. 2). Both medium and pineal were analyzed for melatonin.

Experiment 3

Hamsters and rats were sacrificed during the day in order to obtain the pineal, not for assay or incubation, but to weigh the pineal (electronic balance) after removal of surrounding dura mater and blood, and to establish the relationship between pineal and body weight (Fig. 3). This was used to estimate the pineal weight (Table 1) in selected groups of exp. 1 and 2 from their mean body weight, since their pineals had not been weighed. This estimated pineal weight was used to compare the relative amount of melatonin produced in the medium between rats and hamsters in groups stimulated by 10^{-6} M NE (exp. 1, condition B at 0215 h; exp. 2, 10^{-6} M groups).

Experiment 4

Hamsters were injected with NE 1 μg/g body weight subcutaneously in 0.1 ml physiologic saline or with saline alone either at 2000 h at the end of

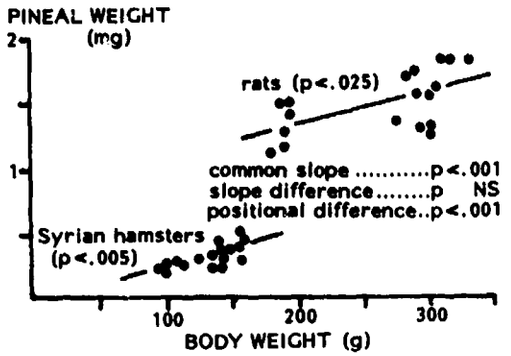

Fig. 3. Single regressions and covariance analysis relating pineal and body weight in adult male rats and Syrian hamsters not used in the incubation or injection experiments.

TABLE 1. Comparison of Rat and Syrian Hamster Melatonin After Pineal Incubation With 10^{-6} M NE in the First Two Experiments, Based on Pineal Weight Projected From the Relationship of Pineal and Body Weight in Other Sets of Animals Shown in the Regression Figure

	Condition	Mean body weight (g)	Projected pineal weight (mg)	Medium melatonin/ projected pineal weight (ng/mg)	Rat/ hamster
Rat	After 20 min light at 6 h into dark	281	1.5	45	
					3.5
Syrian hamster	After 20 min light at 6 h into dark	141	0.35	13	
Rat	Daytime, 4 h into light phase	333	1.65	30	
					3.8
Syrian hamster	After 20 min light at 6 h into dark	160	0.4	8	

the light phase or at 0230 h just after 30 min of light exposure. Other controls received no injections and were sacrificed during the dark phase either in the dark or after 20 min of light exposure. Groups of injected animals were maintained in light or dark until sacrifice at 1 or 2 h after injection (Table 2). Pineals were taken for melatonin assay.

Experiment 5

Separate groups of hamsters were studied during the day or at night, in each case using in vitro (A) as well as in vivo (B) techniques (Table 3, Fig. 4). During the light phase at 1330 h, Syrian hamsters (A) were sacrificed to obtain the pineal for incubation and others (B) were injected. Pineals (A) were incubated with 10^{-6} M NE or desipramine (DMI) alone, both drugs together, or neither. In the case of DMI+NE, the NE was added 10 min after

TABLE 2. In Vivo Injections of NE in Syrian hamsters in the Evening or at Night, in Light or Darkness After the Injections[1]

Set of animals	Condition prior to injection	Time of injection	Condition prior to sampling	Time of sampling	Pineal melatonin (pg/gland) [mean + SE (n)] Control	NE
1	14 h L	2000 h	1 h L	2100 h	66 ± 7 (5)	73 ± 6 (6)
2	14 h L	2000 h	2 h L	2200 h	63 ± 11 (5)	62 ± 8 (6)
3	14 h L	2000 h	1 h D	2100 h	78 ± 11 (6)	83 ± 8 (6)
4	14 h L	2000 h	2 h D	2200 h	73 ± 11 (6)	75 ± 5 (6)
5	—	None	6 h 10 min D	0210 h	1,072 ± 84 (5)	
6	—	None	20 min L	0220 h	120 ± 17 (17)	
7	30 min L	0230 h	1 h L	0330 h	106 ± 7 (12)	107 ± 13 (12)
8	30 min L	0230 h	2 h L	0430 h	103 ± 12 (11)	103 ± 17 (11)
9	30 min L	0230 h	1 h D	0330 h	109 ± 13 (6)	115 ± 21 (6)
10	30 min L	0230 h	2 h D	0430 h	88 ± 11 (6)	124 ± 21 (6)
11	—	None	8 h 40 min D	0440 h	1,225 ± 30 (5)	

[1]Sets 3, 4, 5, 9, 10, and 11 were sacrificed in darkness, the other sets sacrificed (sampled) in light. L, light; D, dark. Norepinephrine (NE, 1 μg/g) or physiologic saline (control) were injected subcutaneously (0.1 ml). Controls in sets 5, 6, and 11 received no injections. There are no significant differences between NE-injected and respective controls in pineal melatonin content.

TABLE 3. Melatonin (6/Group, mean ± SE) After Incubation (A) or After Injection (B) in Syrian Hamsters Studied During the Day (1330 h)[1]

Group	A: in vitro melatonin (ng in medium)	B: in vivo pineal melatonin (pg/gland) at time after injection (h) 0	1	2
Control	0.68 ± 0.11	94 ± 5	82 ± 7	84 ± 11
NE	0.79 ± 0.16	—	89 ± 7	77 ± 8
DMI	1.09 ± 0.31	—	93 ± 9	88 ± 12
NE + DMI	1.54 ± 0.31	—	30 ± 3*	93 ± 9

[1]Drugs in the medium (A) were 10^{-6} M. In the other animals (B), controls received saline (except those at time zero, which received no injection) and the other groups received 1 μg/g NE and/or 5 μg/g DMI. No significant differences among the groups in A were seen.
*$P < 0.001$ vs each of the other groups at 1 h in B. Compare these results with those in Figure 4.

adding the DMI, and the 4-h incubation was timed from the addtion of DMI. Melatonin was determined in the medium. For injected hamsters (B), injections (0.1 ml subcutaneously) were 5 μg/g DMI and/or 1 μg/g NE, or physiologic saline. Each animal received two injections 10 min apart, the first being either saline or DMI and the second either saline or NE, with NE or DMI injected only once in individual animals, some receiving saline and one drug, some both drugs and some only two injections of saline. The animals were sacrificed at 1 or 2 h after the second injection for measurement of pineal melatonin. Incubations (A) and injections (B) using other hamsters were also done using the night with exactly the same protocols described above. Hamsters were brought into the light at 0200 h. Then pineals were taken (A)

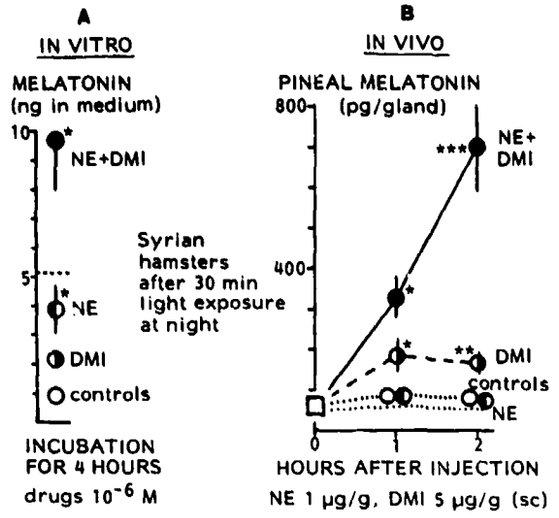

Fig. 4. Melatonin (6/group, mean ± SE) after incubation (A) or after injection (B) in Syrian hamsters studied at night. Light exposure began at 0200 h. Pineals were taken at 0230 h for incubation (A). In other hamsters (B), time zero was 0230 h. In panel A, the dashed line indicates the level of an additive effect of NE and DMI. A two-way analysis of variance indicted an interaction ($P < 0.05$) of NE and DMI. In panel B, controls received diluent (saline, 0.1 ml), and the open square indicates uninjected animals. In either panel, for comparison of means of a group vs controls or, in the case of NE±DMI, comparison vs any of the other groups (at the same time point for panel B), p-values were as follows: *,$P < 0.05$, **,$P < 0.01$, ***,$P < 0.001$.

at 0230 h for incubation with NE, DMI, both, or neither, or animals (B) received injections of saline or DMI 10 min prior to the injection of saline or NE (second injection at 0230 h) and remained in the light until sacrifice 1 or 2 h later. At the time of the injections (B), either during the day or night, uninjected hamsters were also sacrificed to determine basal pineal melatonin content.

RESULTS

Experiment 1

An in vitro response (as melatonin in the medium) with 10^{-6} M NE was significant for Syrian hamster pineals taken in the dark at 0215 h and was abolished by prior exposure of the animals to 6 h but not 20 min of light just before sacrifice (Fig. 1, top panel). In fact, the response appeared augmented after 20 min of light compared to that after sacrifice in the dark because of lowering of the control values by the light exposure prior to sacrifice. No response to 10^{-6} M NE occurred from pineals taken at the end of the light phase (1945 h), or earlier in the light phase at 0845 h, in which values even with 10^{-4} M NE were not significantly different from those of controls. At 45 min into the light phase (0645 h), only the higher NE dose was tested, and the response was significant. In contrast to hamster pineals, rat pineals responded to 10^{-6} M NE at the end of the light phase (Fig. 1, bottom panel). As with hamsters, rat pineals taken at night responded to 10^{-6} M NE.

Experiment 2

Like rat pineals taken during the day (1000 h), hamster pineals taken during the night (0215 h) after 20 min of light exposure responded in a dose-related fashion to NE (Fig. 2). In this experiment, pineal melatonin content at the end of the incubation was also measured. For both species, the great majority of the melatonin produced (92–96% for rats and 95–97% for hamsters) emerged in the medium. However, rat pineals made more melatonin than hamster pineals.

Experiment 3

We further assessed the disparity of in vitro melatonin production between rat and hamster. In animals not used in the other experiments, pineal and body weights were significantly correlated in rats and hamsters with greater pineal weight for the body weight in rats compared to hamsters (Fig. 3). From the mean body weight of the animals from exp. 1 and 2 whose pineals responded to 10^{-6} M NE, an estimated (projected) mean pineal weight was obtained using the relationships in Figure 3. The mean quantity of melatonin in the medium after incubation divided by the projected mean pineal weight indicated that rat pineals appeared to make an estimated three to four times more melatonin per milligram of gland weight than did Syrian hamster pineals when incubated with 10^{-6} M NE (Table 1). Division of the amount of melatonin in the medium by the body weight in these groups also indicates seven- to eightfold more melatonin per gram body weight in the rats than in the hamsters (not shown).

Experiment 4

In order to investigate in hamsters whether night phase injection or the condition of darkness after injection until sacrifice would allow a response of pineal melatonin content to administration of 1 μg/kg NE, pineal melatonin was determined following 1 or 2 h of light or darkness, after injection either at the end of the light phase (2000 h) or at 0230 h. In the latter case (0230 h injections), the animals received 30 min of light exposure prior to injection to reduce basal pineal melatonin and provide a condition similar to that in which hamsters' pineals taken at night responded to NE alone in vitro (exp. 1, 2, and 5). In no case did pineal melatonin respond to this dose of injected NE (Table 2).

Experiment 5

Mid-daytime incubation of Syrian hamster pineals with NE and/or DMI did not produce significant effects of melatonin in the medium (Table 3). Daytime injection of NE and/or DMI produced no effects on pineal melatonin except for depressed melatonin after NE+DMI at 1 h, not evident at 2 h (Table 3). The presence of DMI in the medium significantly enhanced the nocturnal in vitro NE response of the hamster pineal (Fig. 4, panel A). The small effect of DMI alone was not statistically significant. Injection of DMI at night resulted in a small but significant in vivo rise of pineal melatonin; whereas, NE injection alone was not effective (Fig. 4, panel B). However,

injection of DMI allowed a dramatic response of hamster pineal melatonin to injection of NE at night.

DISCUSSION

Changes in sensitivity of the rat pineal to beta-adrenergic stimulation during the 24-h cycle previously have been studied in vitro and in vivo with use of isoproterenol and measurement of the response in pineal N-acetyltransferase (NAT, which determines melatonin synthesis) [Klein and Weller, 1973]. These results have been reviewed [Romero, 1976] and indicate that the pineal is most sensitive after prolonged light exposure of the rats and becomes progressively less sensitive at night, due to beta-receptor down-regulation from the nocturnal surge of transmitter (NE) release. Though we did not observe evidence of a reduced response at night in rat pineals in our system using one dose of NE (10^{-6} M) as the stimulus in the medium and melatonin as the response, we did see that the rat pineal was sensitive to this dose in vitro during and at the end of the light phase. Syrian hamsters appear to be quite different. Their pineals have a rhythm of sensitivity to NE, involving nocturnal development of sensitivity to NE potentiated by DMI, and relative insensitivity during the day. Prolongation of the light phase prevented nocturnal development of an in vitro hamster response to 10^{-6} M NE, indicating that prolonged light exposure has a negative influence on pineal sensitivity to NE in this species.

Using rat pineals, Parfitt and Klein [1977] and Sartin et al. [1978] found stimulation of ^{3}H-melatonin accumulation in the medium (synthesized from ^{3}H-tryptophan) after incubation for 6–10 h with 10^{-6} M NE. Wilkinson and Arendt [1978] found stimulated accumulation of RIA-determined melatonin in the medium of rat pineals incubated 2–3 h with 10^{-6} M NE or 5 (10^{-9}) M isoproterenol. Craft and Reiter [1984] obtained Syrian hamster pineals 4–5 h into the light phase and cultured them for 24 h. Then, after 5 h incubation with 0.3 mM NE, they noted a rise in ^{3}H-melatonin (from ^{3}H-serotonin) in the incubated pineal tissue. The influence of such long-term culture on the rhythm of sensitivity of hamster pineals to NE is not known. We now extend these observations to show that short-term (4 h) incubation of pineals and direct measurement of unlabeled melatonin in the medium can be employed to investigate noradrenergic control of melatonin synthesis in the rat and Syrian hamster.

In both species, dose-related rises in melatonin after incubation with NE were similar in the pineal and medium, with the great majority of the melatonin formed appearing in the medium. This essentially represents melatonin synthesized during incubation, because glands incubated even without NE produced many-fold more melatonin in the medium than is present in glands measured without incubation. Pineals from hamsters taken during the day, or at night after brief exposure to light, delivered into the medium approximately 3 pg/min without NE and about 19 pg/min at night with a maximally effective dose of NE. This compares favorably with in vivo melatonin secretory rates calculated by Rollag et al. [1980] in hamsters from pineal melatonin disappearance rates and the pineal melatonin content pro-

file: 1.6–7 pg/min during the day, an average 23 pg/min during the first 4 h of the nocturnal surge, and 45 pg/min in the 4 h leading up to the nocturnal peak. Our in vitro model appears relevant to the in situ pineal gland and indicates the likelihood that NE is the neurotransmitter responsible for the nocturnal melatonin surge in the Syrian hamster.

Rat pineals make more melatonin in vitro under NE stimulation than do hamster pineals, and this is partially explained by their larger pineal and body size. However, it appears that even after correction for estimated pineal weight or for body weight, rat glands made several-fold more melatonin per milligram of pineal or per gram of body weight than did those of hamsters incubated with the same NE concentration. These results may provide physiologic relevance for the finding [Craft et al., 1985] of a several-fold greater density of beta-receptors in rat than in hamster pineal.

Daytime rat pineal NAT rose after subcutaneous injection of 1 μg/g NE or isoproterenol, and this response was much greater if superior-cervical-ganglionectomy (SCGX, pineal denervation) had previously been performed [Deguchi and Axelrod, 1973]. In contrast, isoproterenol injection reduced or did not alter daytime pineal melatonin content in intact Syrian hamsters and had no effect in SCGX hamsters [Lipton et al., 1982]. Hourly injections of 0.5–1 μg/kg NE, beginning at the end of the normal light phase (with the experiments continuing in the light), elevated pineal melatonin to the normal nocturnal level in Djungarian hamsters but had no effect in Syrian hamsters [Steinlechner et al., 1984]. Because those animals were in light after the injections and prolonged light exposure appeared to inhibit the in vitro response to NE in the present studies, we determined whether putting the Syrian hamsters in darkness between injection and sacrifice would allow an in vivo pineal melatonin response to a single injection of 1 μg/kg NE. It did not, even at the time of night at which pineals responded in vitro, which suggested that some factor other than light exposure also prevented an in vivo response to circulating (injected) NE.

We found that this factor is likely to be catecholamine uptake in the pineal sympathetic nerve endings, a factor already shown partially to protect pinealocytes in rats from stimulation by circulating catecholamines in studies using DMI to block uptake [Parfitt and Klein, 1976]. Although other actions of tricyclic agents have been described, the main effect of DMI in neural tissue is potent and specific blockade of uptake of adrenergic agents such as NE [Aberg-Wistedt et al., 1984; Manias and Taylor, 1983; Ross and Renyi, 1975; Iversen, 1971]. Further, Parfitt and Klein [1976] found that its role in permitting catecholamine action on rat pineal NAT involves blocking neuronal uptake of beta-acting catecholamine and not a mechanism involving some other transmitter. They found that DMI potentiated the effect of epinephrine in vitro only if the nerve endings were left intact. In pineals with nerve endings, DMI did not potentiate the effect of isoproterenol, an adrenergic agent itself resistant to uptake. If the potentiating effect of DMI had been through a nonadrenergic transmitter, it would have been expected to occur also in the latter case. In our Syrian hamsters at night with pineal melatonin lowered by a brief exposure to light, injection of DMI allowed a dramatic effect of NE injection. Incubation of pineals from this species at

night with DMI also potentiated the effect of NE in the medium, indicating that the nerve endings retain some of their protective uptake function during incubation.

That NE by itself is effective in vitro at night suggests that this condition allows a high enough local NE concentration to partially overcome the effect of neuronal uptake or perhaps that uptake is less active in vitro. Interestingly, rat pineals taken during the day and incubated in similar conditions (drugs 10^{-6} M) also exhibited some in vitro neuronal uptake function because the presence of DMI potentiated the effect of NE on accumulation of ^{3}H-melatonin (formed from ^{3}H-tryptophan) in the medium [Parfitt and Klein, 1977]. In contrast, we found no significant response of Syrian hamster pineals to NE at midday in the presence of DMI, apparently reflecting the difference between these two species in daytime pineal sensitivity.

The human pineal gland may also demonstrate uptake protection. In depressed patients, DMI administration augmented the nocturnal surge of plasma melatonin (presumably from enhanced local NE neurotransmission), though DMI was not combined with exogenous or stimulated humoral catecholamine during the day [Arendt, 1985].

The in vitro response to NE at 0645 h may represent nocturnal sensitivity continuing briefly into the light phase. Whether a higher injected NE dose might stimulate in vivo hamster melatonin at night without use of DMI is not yet known. Further, our lack of daytime stimulation of NE might represent a function of dose and of time after NE exposure in view of the nonsignficant trend toward a small rise of medium melatonin after 4 h incubation with NE+DMI, and the return of the depressed pineal melatonin value (at 1 h after injection of NE+DMI) to baseline by 2h in the daytime study (Table 3). Use of higher doses of NE and/or sampling later after initial daytime exposure to NE+DMI might conceivably disclose a melatonin response and indicate that the reduced daytime insensitivity of the pineal in this species is only relative with respect to that at night and to that in the rat. Nevertheless, our results comparing the hamster melatonin response between day and night included four conditions with respect to NE (with and without DMI, both in vitro and in vivo) and indicated a clear rise in sensitivity during the night. Whether this change is intrinsic to the pinealocytes, is due to induction of receptor or postreceptor mechanisms by the nocturnal rise in local endogenous sympathetic stimulation, or stems from a selective prejunctional mechanism such as primary nocturnally reduced effectiveness of neuronal uptake or secondary reduced neuronal uptake of added NE competing for uptake disposal with greater amounts of local NE normally released during the night is not yet known with certainty. However, it appears that daytime hamster pineal insensitivity results partly from changes of the pinealocytes, because isoproterenol (3 μg/g) did not stimulate pineal melatonin content for up to 5 h after daytime injection in intact or even SCGX Syrian hamsters [Lipton et al., 1982].

The marked rhythm in pineal sensitivity to its presumed neurotransmitter in the Syrian hamster (sensitive at night) found in our studies explains the report [Steinlechner et al., 1985] of the light-induced fall in pineal melatonin at night being prevented in this species by an injection of isoproterenol,

which is relatively resistant to neuronal uptake. A fall in pineal melatonin during the day after isoproterenol [Lipton et al., 1982] or NE+DMI (present exp. 5) is not yet explained. The initially reported small rise in hamster pineal melatonin after daytime injection of isoproterenol [Tamarkin et al., 1979] was not confirmed later in a more extensive study [Lipton et al., 1982].

Craft et al. [1985] found beta-receptors in the Syrian hamster pineal that (unlike in the rat) did not rise in density after prolonged light exposure or SCGX and noted that hamster pineal NE synthesis (evaluated from L-dopa accumulation after decarboxylase inhibition) normally rose at night. The nocturnal pineal melatonin rise in this species is prevented by beta-blockade [Lipton et al., 1981] and SCGX [Panke et al., 1979; Vaughan and Reiter, 1985]. In the light of such findings, the nocturnal appearance of a pineal melatonin response to NE and protection from humoral-route activation by a neuronal uptake system in Syrian hamsters (a) indicate that NE is likely the pineal neurotransmitter responsible for the nocturnal melatonin surge, (b) explain previous negative results with NE, and (c) provide possible mechanisms to eliminate noise in the 24-h rhythmic melatonin signal by preventing untimely rises in melatonin synthesis due to general sympathetic stimuli. Indeed, such partitioning of sympathetic function for independent and specific control of the pineal may help explain the observed lack of evidence for augmented melatonin production after general sympathetic stimuli in Syrian hamsters and humans [see Vaughan, 1984; Gupta et al., 1983; Champney et al., 1985; Vaughan et al., 1985]. Pineal neuronal uptake might afford protection against the humoral limb of a general sympathetic response. Relative daytime pinealocyte insensitivity in terms of melatonin production also remains a possiblity and might protect against nonspecific activity in the sympathetic neuronal pathway to the pineal (if such occurs) in a general sympathetic response. Available information does not yet completely explain the mechanisms for reduced Syrian hamster pineal sensitivity to exogenous NE during the day or after prolonging light exposure into one night.

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