Evidence that Rat Pineal Thyroxine 5′-Deiodinase is Primarily Stimulated by β- and not α-Adrenergic Agonists and that Its Adrenergic-Stimulated and Spontaneous Rhythmic Nocturnal Rise Require RNA and Protein Synthesis (42849)

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Abstract. **Pineal thyroxine 5′-deiodinase (5′-D) activity rose >10-fold above the basal level 2–3 hr after 1 mg/kg isoproterenol and returned to near the basal level by 6 hr. The same dose of norepinephrine or phenylephrine was without effect, but phenylephrine modestly potentiated isoproterenol-stimulated 5′-D activity. Either actinomycin D or cycloheximide treatment markedly decreased diurnal isoproterenol stimulation and the spontaneous rhythmic nocturnal rise of pineal 5′-D. The data indicate that pineal 5′-D activity is very similar to pineal serotonin *N*-acetyl transferase in being primarily stimulated by β-adrenergic agonists and requiring new RNA and protein synthesis for its activation.** [P.S.E.B.M. 1989, Vol 190]

Thyroxine (T_4) is converted to the biologically active 3,5,3′-triiodothyronine (T_3) by T_4 5′-deiodinase (5′-D), which has two main subgroups. Type I is present in liver and kidney and many other organs. Type II is found in a limited number of organs such as the central nervous system, pituitary, brown adipose tissue, and pineal and Harderian glands in the rat (1–4). Type II is characterized by a lower K_m for T_4, relative insensitivity to inhibition by 6-*n*-propyl-2-thiouracil, and a marked increase in its activity in hypothyroidism. Type II 5′-D is considered to play a role in local conversion, whereas Type I contributes to plasma T_3.

In the rat pineal gland, there is a nyctohemeral rhythm in Type II 5′-D activity with a zenith at midnight approximately 20-fold above the diurnal value (4, 5). This nocturnal rise is markedly inhibited by propranolol and depends on intact superior cervical ganglia (4, 6) and the daytime enzyme activity is increased by isoproterenol (5), indicating an important role of β-adrenergic stimulation.

In this study, we determined the time course of the increase in 5′-D induced by isoproterenol and examined the effect of inhibition of RNA and protein synthesis on the nocturnal rise and isoproterenol stimulation of pineal 5′-D activity to evaluate the mechanism of activation of this enzyme.

Materials and Methods

Animals. Adult male Sprague-Dawley rats (200–250 g) were obtained from Simonsen. The rats were maintained three per cage on a 12-hour light-dark schedule (light on: 0600–1800 hr) with free access to Purina Lab Chow and water. Rats were allowed to acclimate to these conditions for at least 2 weeks before they were used.

Chemicals. T_4, T_3, DL-dithiothreitol, L-isoproterenol, L-phenylephrine, *l*-norepinephrine, actinomycin D, and cycloheximide were purchased from Sigma (St. Louis, MO); 6-*n*-propyl-2-thiouracil was purchased from ICN Biochemicals (Cleveland, OH). $Na^{125}I$ was purchased from Amersham. [3′,5′-^{125}I]T_4 was labeled from T_3 in our laboratory by the chloramine T method (7).

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Experimental Procedures. For injection experiments, all of the drugs were dissolved in saline, and control groups received the same volume of saline vehicle. Rats were killed by decapitation. Pineal glands were rapidly removed, frozen, and stored at −70°C until assayed. All experiments were performed at least twice with essentially identical results each time, except that the first study (temporal response to isoproterenol) was performed only once.

Assay of 5′-D Activity. Each pineal gland was homogenized separately in 100 m*M* potassium phosphate buffer (pH 7.0) containing 1 m*M* EDTA and 10 m*M* dithiothreitol (DTT) and centrifuged at 3000 rpm for 15 min. Resulting supernatants were incubated with 2 n*M* of [^{125}I]T_4 in the presence of 1 m*M* EDTA, 10 m*M* DTT, and 1 m*M* 6-*n*-propyl-2-thiouracil for 1 hr at 37°C. Released $^{125}I^-$ was separated and counted as described previously (2, 5). Protein content was determined by Bradford's method (8), and the enzyme activity was expressed as fmoles of I^- released/mg protein/min.

Statistics. Data are presented as the mean ± standard error. Statistical analyses were performed with analysis of variance and the Newman-Keuls multiple comparison test.

Results

Time Course of Isoproterenol Stimulation of Pineal 5′-D. Rats were injected subcutaneously with 1 mg/kg body wt of isoproterenol between 1100 and 1300 hr and were killed by decapitation at various time intervals (0, 1, 2, 3, 4, 6 hr) between 1400 and 1700 hr.

Enzyme activity reached a zenith >10-fold higher than baseline in 2–3 hr, then returned to near the baseline level in 6 hr (Fig. 1). The effect of adrenergic agonists was therefore determined 3 hr after their injection in the following experiments.

Comparison of the Effect of α- and β-Adrenergic Agonists on Pineal 5′-D. Rats were injected subcutaneously with 1 mg/kg body wt of isoproterenol, norepinephrine, phenylephrine, or vehicle between 1100 and 1200 hr and killed 3 hr later. Neither norepinephrine nor phenylephrine changed pineal 5′-D activity significantly ($P > 0.05$), whereas isoproterenol stimulated the enzyme activity as before ($P < 0.01$) (Fig. 2).

Since a potentiating effect of α-adrenergic agonists on β-adrenergic stimulation of pineal *N*-acetyl transferase (NAT) has been reported (9), the combined effect of isoproterenol and phenylephrine was examined. Rats were injected with 0.3 mg/kg body wt of isoproterenol, 1 mg/kg body wt of phenylephrine, or both of these drugs and killed 3 hr later. Again, isoproterenol stimulated pineal 5′-D activity significantly ($P < 0.01$) and phenylephrine itself was without effect. However, phenylephrine increased isoproterenol-stimulated 5′-D activity slightly but significantly ($P < 0.05$, Fig. 3).

Effect of Actinomycin D or Cycloheximide on Isoproterenol-Stimulated Pineal 5′-D Activity. Isoproterenol (1 mg/kg body wt) or saline was injected subcutaneously at 1200 hr. One hour before the injection, actinomycin D (1 mg/kg body wt), cycloheximide (20 mg/kg body wt), or saline was injected intraperitoneally. The rats were killed 3 hr after isoproterenol injection.

Actinomycin D blunted or abolished isoproterenol-stimulated pineal 5′-D activity ($P < 0.01$), but did not change basal enzyme activity significantly ($P > 0.05$, Fig. 4). Cycloheximide abolished iosproterenol-stimulated pineal 5′-D activity ($P < 0.001$) and also did not change basal enzyme activity significantly ($P > 0.05$) (Fig. 5).

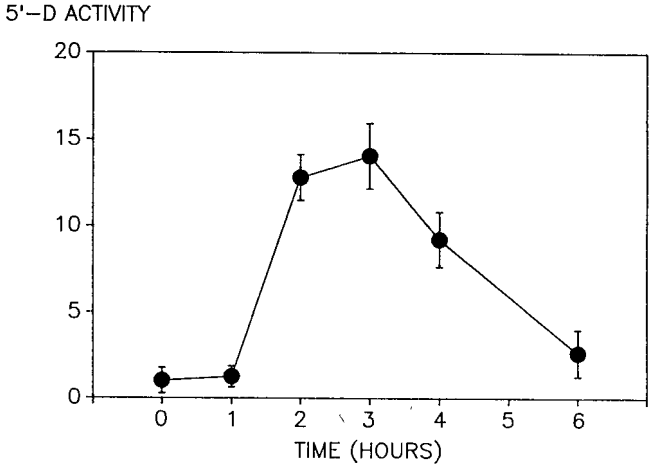

Figure 1. Time course of pineal 5′-D activity after 1 mg/kg of sc isoproterenol. 5′-D activity is expressed in this and the subsequent figures as fmoles I^- released/mg protein/min. Each point and vertical line represent the mean ± SE of the enzyme activity determined in five rats. Values at 2, 3, and 4 hr were significantly higher than at 0 hr ($P < 0.01$).

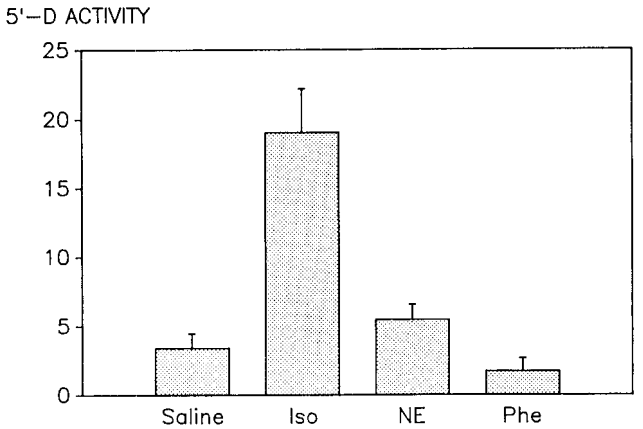

Figure 2. Effect of 1 mg/kg of sc isoproterenol (Iso), sc norepinephrine (NE), or sc phenylephrine (Phe) on pineal 5′-D activity, expressed as in Figure 1. In this and subsequent graphs, each bar and vertical line indicate the mean ± SE of the enzyme activity determined in each group. In this experiment there were four to five rats in each group. Only the isoproterenol-injected rats had 5′-D significantly higher than the saline-injected controls ($P < 0.01$).

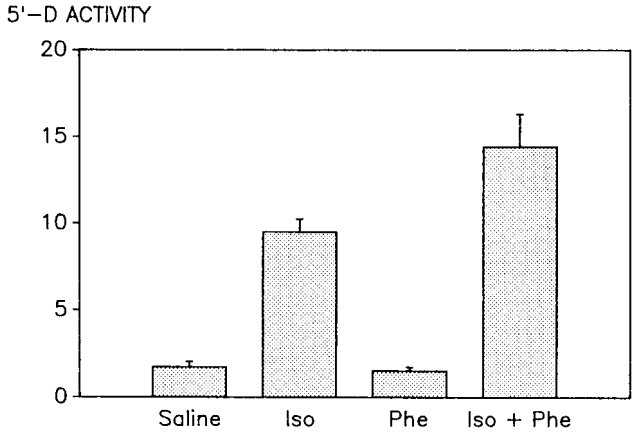

Figure 3. Effect of 0.3 mg/kg of sc isoproterenol (Iso), 1 mg/kg of sc phenylephrine (Phe), or a combination of these drugs on pineal 5′-D activity. There were five rats in each group. The Iso and Iso + Phe groups were significantly higher than the saline group ($P < 0.01$) and the Iso + Phe group was higher than the Iso group $P < 0.05$.

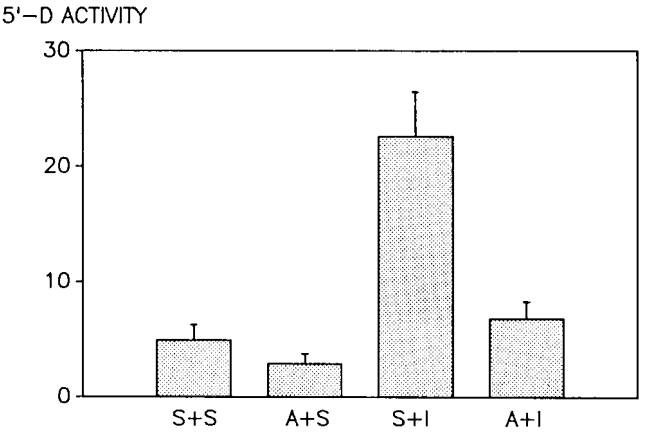

Figure 4. Effect of actinomycin D (A) on isoproterenol (I) stimulation of pineal 5′-D activity. Actinomycin D (1 mg/kg ip) or saline (S) was injected at 1100 hr, 1 hr before the injection of L-isoproterenol (1 mg/kg sc) or saline (S). 5′-D activity is expressed as in Figure 1. There were five rats in each group. S + I was significantly higher than the other three groups ($P < 0.01$), which were not different from each other ($P > 0.05$).

Effect of Actinomycin D or Cycloheximide on the Nocturnal Rise of Pineal 5′-D Activity. Based on the findings described above, we tested the possibility that actinomycin D and cycloheximide would inhibit the spontaneous nocturnal rise of pineal T_4 5′-D activity. Actinomycin D (1 mg/kg body wt), cycloheximide (20 mg/kg body wt), or saline was injected intraperitoneally shortly before the onset of darkness. The rats were killed by decapitation at 2400 hr. Daytime control rats were killed at 1200 hr. As reported previously (5), pineal 5′-D activity was approximately 20-fold higher at midnight than at noon in the control rats ($P < 0.01$). Both actinomycin D and cycloheximide pretreatment abolished this nocturnal rise of pineal 5′-D ($P < 0.01$, Fig. 6).

Discussion

Light information and rhythmic signals are transmitted to the pineal by the release of norepinephrine at terminals of nerves originating from the superior cervical ganglia. NAT, which catalyzes the acetylation of serotonin to the immediate precursor of melatonin, *N*-acetylserotonin, has a dramatic nyctohemeral rhythm reaching its zenith at midnight and is primarily, if not solely, regulated by a β-adrenergic mechanism (10). Pineal 5′-D has a similar nyctohemeral rhythm with a zenith at midnight (4, 5). This nocturnal rise is depressed by the β-adrenergic antagonist, propranolol, and its low diurnal activity is stimulated by the β-

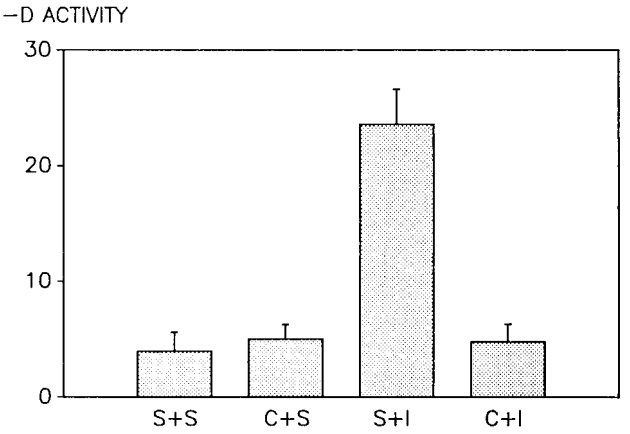

Figure 5. Effect of cycloheximide (C) on isoproterenol (I) stimulation of pineal 5′-D activity. Cycloheximide (20 mg/kg ip) or saline (S) was injected at 1100 hr, 1 hr before the injection of isoproterenol (1 mg/kg sc) or saline (S). 5′-D activity is expressed as in Figure 1. There were five to six rats in each group. S + I was significantly higher than the other three groups ($P < 0.001$), which were not different from each other ($P > 0.05$).

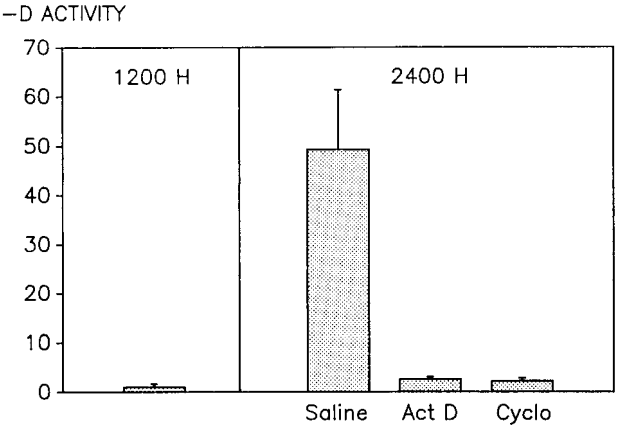

Figure 6. Effect of actinomycin D (Act D) or cycloheximide (Cyclo) on the nocturnal rise of pineal 5′-D activity. Actinomycin D (1 mg/kg), cycloheximide (20 mg/kg), or saline was injected intraperitoneally shortly before the onset of dark (1800 hr). Control rats were killed at 1200 hr and injected rats were killed at 2400 hr. 5′-D activity is expressed as in Figure 1. There were five rats in each group. The saline 2400-hr group was significantly higher than the other three groups ($P < 0.01$), which were not significantly different from each other.

adrenergic agonist, isoproterenol (5). In the present study this regulatory mechanism was further investigated.

We have studied only pineal 5′-D and NAT rhythms in our laboratory. Although both have similar high amplitude rises at approximately midnight, they are not identically controlled. NAT falls dramatically from its nocturnal peak within a few minutes of acute light exposure whereas 5′-D does not and both the spontaneous nocturnal zenith and decline to baseline of 5′-D occur significantly earlier than that of NAT when both are measured in the same animals (11). The pineal contains many enzymes and not even all of those critical to melatonin synthesis have the same striking nyctohemeral rhythm of 5′-D and NAT (12). Thus, it is probable that the controlling mechanisms for fluctuations in 5′-D activity are not nonspecifically related to general pineal metabolism but have at least some measure of independent activation.

The temporal profile of isoproterenol stimulation was initially studied. The enzyme activity reached a maximal level in 2–3 hr and returned to near the baseline level within 6 hr after isoproterenol injection. In contrast, the α-adrenergic agonist, phenylephrine, itself did not stimulate 5′-D activity significantly, indicating that a β- not an α-adrenergic mechanism is primarily responsible. However, phenylephrine slightly increased isoproterenol-stimulated 5′-D activity, suggesting a potentiating role of an α-adrenergic mechanism on β-adrenergic stimulation of 5′-D activity. Klein *et al.* (9) reported that an α-adrenergic mechanism protentiates β-adrenergic activation of NAT *in vivo* and in cultured pineal glands. A similar mechanism is apparently involved in 5′-D activation.

Norepinephrine did not stimulate 5′-D activity in the present study nor does it markedly alter pineal NAT activity (13). The ineffectiveness of norepinephrine can be explained by rapid inactivation of this compound, presumably due to the uptake of catecholamines by the sympathetic nerve endings in the pineal (14).

The time course of stimulation of the low basal NAT activity during the light period or after 6 hr of additional light by isoproterenol (9, 15) or L-dopa (13) is similar to our present result for 5′-D. Both enzymes have a similar lag time of response of 1–2 hr. Actinomycin D and cycloheximide markedly decreased isoproterenol-stimulated and the rhythmic nocturnal rise of NAT (15, 16), suggesting that new RNA and protein synthesis are required. There is no lag period in the reinduction of NAT from basal levels at midnight after a short light exposure (15); this reinduction is inhibited by cycloheximide but not by actinomycin D treatment (16), suggesting that the lag period may be due to the time required for new RNA synthesis.

On the basis of the similarity of the lag period after isoproterenol injection between NAT and 5′-D, it appeared likely that a similar mechanism might be involved in 5′-D stimulation. In the present study, both actinomycin D and cycloheximide pretreatment markedly decreased isoproterenol stimulation of 5′-D activity, suggesting the involvement of new RNA and protein synthesis in this activation. The presence of a lag period before the nocturnal rise of 5′-D after the onset of dark (5) and the inhibition of the nocturnal rise of 5′-D activity by pretreatment with actinomycin D or cycloheximide in the present study further support this concept. Our data do not exclude the further possibility that the rise in 5′-D activity is caused by activation of preexisting 5′-D by an activating enzyme whose operation requires gene expression and protein synthesis.

Neural regulation of brown adipose tissue 5′-D has also been studied (17). Brown adipose tissue 5′-D is markedly activated by cold acclimation through an α_1-adrenergic mechanism (17). It is of interest that cold-induced stimulation of brown adipose tissue 5′-D has a lag period of 1–2 hr (18), and both cold- and norepinephrine-induced stimulation is also abolished by actinomycin D or cycloheximide (18, 19), similar to our observations on pineal 5′-D.

Our present study suggests that daytime β-adrenergic stimulation and the rhythmic nocturnal rise of pineal 5′-D activity is not due to activation of preexisting enzyme, but to stimulation of RNA and protein synthesis. However, there may be nonspecific toxic effects of these potent drugs and our data do not specifically distinguish between an inhibition of enzyme formation or an acceleration of enzyme degradation. From the known actions of the drugs employed, the latter seems unlikely. An unambiguous conclusion will have to await further data which might most clearly be obtained in an *in vitro* pineal cell system.

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