In Vivo Stimulation of Rat Pineal Type II Thyroxine 5′-Deiodinase Activity by Either Norepinephrine or Isoproterenol (43099)

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Abstract. **Herein we show, for the first time, a very marked increase in thyroxine 5′-deiodinase (5′-D) activity in rats injected with norepinephrine (NE) and desmethylimipramine, a drug which inhibits NE uptake by nerve terminals. The response to NE was greater in pineals collected from hypothyroid animals than in glands from euthyroid animals. NE was more effective in stimulating pineal 5′-D than was isoproterenol, suggesting that, in addition to β-adrenergic receptors, α-adrenergic receptors might be involved in the 5′-D activation. However, phenylephrine, an α-adrenergic agonist, did not potentiate the effect of isoproterenol on pineal 5′-D activity. The nocturnal increase in pineal 5′-D activity was completely abolished by propranolol, a β-adrenergic receptor blocker, while prazosin, an α-adrenergic receptor blocker, had minimal effect. These results show that the role of α-receptors in promoting the NE-mediated rise in rat pineal 5′-D activity is minor in contrast to the role of β-adrenergic receptors.**

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Rhythms in thyroxine type II 5′-deiodinase (5′-D) activity have been described in both the rat pineal (1, 2) and the rat Harderian gland (3), where rhythms in melatonin production also have been reported (4, 5). The enzyme had been previously found in a small number of tissues, namely, in the anterior pituitary gland (6), brain (7), brown adipose tissue (8), placenta (9), and epidermal keratinocytes (10). 5′-D Is believed to have an important role in maintaining the intracellular levels of triiodothyronine (T_3), serving as a defense against thyroid hormone deficiency. The most important regulatory factor for this isoenzyme is the level of circulating T_3, exhibiting an important increase in its activity during hypothyroidism and a marked inhibition in the presence of thyroxine (T_4) (11, 12). In brown adipose tissue, an additional regulatory mechanism has been described since acute cold stress activates the enzyme through an increase of the adrenergic input mediated by α_1-receptors (13).

In the rat pineal gland, besides the thyroid status, 5′-D activity is also regulated by the light:dark cycle (see 14 for review); 5′-D activity exhibits a progressive rise in activity after the onset of the dark period and reaches a peak value 5–6 hr later; the peak coincides with the peak values described for both pineal melatonin content and *N*-acetyltransferase activity (2, 15, 16). The nocturnal increase in 5′-D activity seems to be dependent on the sympathetic noradrenergic input since either continuous light exposure or superior cervical ganglionectomy prevents it (2, 17, 18). Additionally, both *in vivo* and *in vitro* studies have shown that isoproterenol, a β-adrenergic agonist, also activates the 5′-D activity, while propranolol, a β-adrenergic blocker, inhibits it (17, 19–22).

In the present study we show, for the first time, the *in vivo* activation of rat pineal 5′-D activity by injecting norepinephrine (NE), the physiologic neurotransmitter released by the sympathetic nerve endings within the pineal gland, at night. The study also examines the relative roles of β- and α-adrenergic receptors in mediating the rise in pineal 5′-D activity.

Materials and Methods

Male Sprague-Dawley rats, weighing approximately 100 g, were purchased from Harlan (Indianapolis, IN) and allowed to acclimate to the animal facilities. Animals received food and water *ad libitum* and were exposed to an automatically regulated light:dark

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cycle of 14:10; the lights were turned off daily from 20:00 through 06:00 hr. When required, animals were rendered hypothyroid by adding 20 mg/100 ml methimazole to the drinking water; this treatment was maintained for 7 or 8 days before the animals were used for the experiments. On the day of the experiments, animals were killed by decapitation and pineals quickly collected, frozen on solid CO_2, and stored at −70°C until assayed for 5′-D activity.

The measurement of 5′-D activity was based on the release of radioiodine from T_4. This activity is specific for type II 5′-D, since the substrate contains ^{125}I only in position 5′-D. Other deiodinating activities, i.e., conversion of T_4 to rT_3, would release only nonradioactive iodide (15). Briefly, pineals were disrupted by ultrasound in 100 μl of cold 0.05 *M* phosphate buffer (pH 6.8). Then 50 μl were immediately incubated in the presence of 40 m*M* dithiothreitol and 2 n*M* [3′,5′-^{125}I]T_4 as substrate (200 μl of final volume). The substrate concentration was similar to the K_m value described for 5′-D activity in rat pineal (1). Reaction was started by the addition of the substrate and continued for 60 min at 37°C. Control incubations were performed by omission of the homogenates. The reaction was terminated by the addition of 100 μl of cold 2% bovine serum albumin and 750 μl of 10% trichloroacetic acid. The samples were centrifuged for 10 min at 3000 rpm and the supernatant was decanted onto a 1.5-ml column packed with Dowex-50W ion exchange resin and eluted with 2 ml of 10% glacial acetic acid (23). Radioactivity in the eluate, corresponding to the ^{125}I released, was counted in a gamma counter as an index of 5′-D activity. The recovery of ^{125}I in this process was better than 95%. Specific enzymatic activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. 5′-D Activity is referred to as femtomoles of ^{125}I released per gland per hour. Results are expressed as mean ± SE. Significant differences between groups were determined by Student's *t* test.

All reagents were of analytical grade and obtained from commercial sources. T_3, DL-dithiothreitol, (−)-isoproterenol (ISO), (−)-norepinephrine (NE), L-phenylephrine (PE), prazosin (PRAZ), DL-propranolol (PROP), and desmethylimipramine (DMI) were purchased from Sigma (St. Louis, MO); NA^{125}I was purchased from Amersham (Arlington Heights, IL). ^{125}I was bound to T_3 using the chloramine T method, as described elsewhere (24), and purified through a 3-ml Sephadex LH-20 column. The purified tracer contained less than 2% free iodine and was immediately used for 5′-D analyses.

Experiment 1. Fifty-six rats (eight groups of seven each) were used in this study. Half of the animals were rendered hypothyroid to increase the response of rat pineal 5′-D activity to β-adrenergic agonists (16). On the night of the experiment, groups of both euthyroid and hypothyroid animals were maintained under continuous light (to suppress the darkness-induced rise in pineal 5′-D activity) and injected (sc) with either saline, ISO (a β-adrenergic receptor agonist) (1.0 mg/kg body wt), NE (1.0 mg/kg body wt) + DMI (a NE re-uptake blocker) (5 mg/kg body wt), or DMI (5 mg/kg body wt) at each of the following times: 20:00, 22:00, and 00:00 hr. The animals were subsequently killed by decapitation at 01:00 hr and pineals were quickly collected and frozen on solid CO_2. In this and subsequent studies the animals were killed under a dim red light.

Experiment 2. Twenty-four euthyroid animals (three groups of eight each) were used in this study. On the night of the experiment, all of the groups of animals were maintained under continuous light and injected (sc) with either saline, ISO (1.0 mg/kg body wt), or ISO (1.0 mg/kg body wt) + PE (an α-receptor agonist) (1 mg/kg body wt) at 20:00, 22:00, and 00:00 hr. The animals were killed at 01:00 hr and pineals collected for 5′-D activity determinations.

Experiment 3. Twenty-four hypothyroid animals (three groups of eight each) were used in this study. On the day of the study, the animals were permitted to enter the normal dark period at 20:00 hr; thereafter, the three groups of animals were injected (sc) with either saline, PRAZ (an α-adrenergic receptor antagonist) (20 mg/kg body wt) at 20:00 hr. All animals were killed at 01:00 hr under a dim red light and pineals were quickly collected.

In all three experiments, the drug dosages used were consistent with those employed in previous studies in which pineal 5′-D activity was modified (15, 19–22). The drugs were given at three time points so that the glands would be continuously exposed to the drugs; tissues were collected at 01:00 hr (5 hr after drug administration onset) because this is the approximate time at which maximal pineal 5′-D activity is attained when animals are exposed to darkness (14).

Results

Experiment 1. In this experiment, the *in vivo* effect of either NE plus DMI or ISO administration alone on 5′-D activity was tested. Administering either ISO or NE plus DMI clearly increased pineal 5′-D activity in both euthyroid and hypothyroid animals (Fig. 1). However, the response of the hypothyroid animals to the drugs was greater; even basal values of 5′-D activity, in the absence of drugs, were enhanced by hypothyroidism. The effect of NE plus DMI on 5′-D activity seemed to be somewhat more potent than that which follows ISO administration. DMI alone had no effect on the enzyme activity in either euthyroid or hypothyroid animals.

Experiment 2. NE, a combined α- and β-adrenergic receptor agonist, seemed to be more potent than

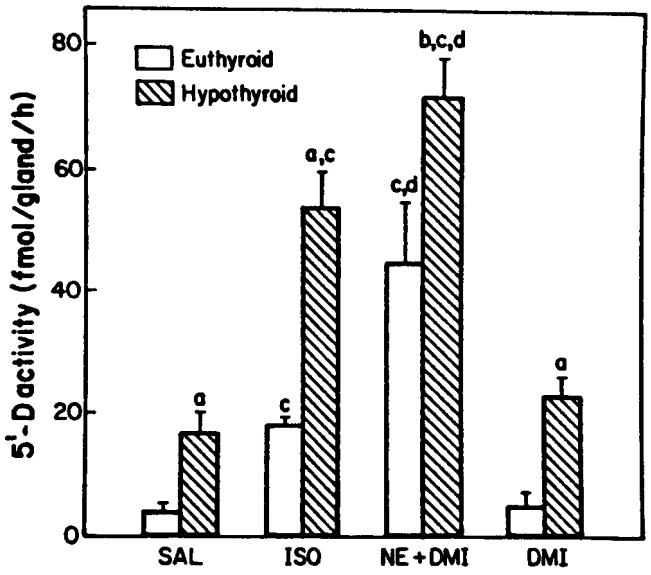

Figure 1. Effect of either ISO, NE + DMI, or DMI alone on pineal 5′-D activity of euthyroid and hypothyroid rats. Rather than entering darkness at 20:00 hr, rats were maintained under light and were repeatedly injected subcutaneously (20:00, 22:00, and 00:00 hr) with either saline (SAL), ISO (1.0 mg/kg body wt), NE + DMI (5 mg/kg body wt), or DMI alone. Animals were killed at 01:00 hr and pineals were collected for 5′-D activity determinations. Results are the mean ± SE of seven animals per group. a, $P < 0.001$ versus euthyroid animals; b, $P < 0.05$ versus euthyroid animals; c, $P < 0.001$ versus SAL; d, $P < 0.01$ versus ISO.

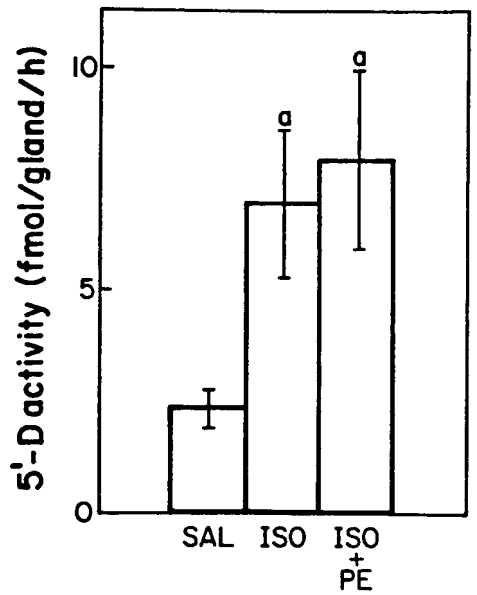

Figure 2. Effect of either ISO or ISO + PE on pineal 5′-D activity in euthyroid rats. Rather than entering darkness at 20:00 hr, animals were maintained under light and were repeatedly injected subcutaneously (20:00, 22:00, and 00:00 hr) with either saline (SAL), ISO (1.0 mg/kg body wt), or ISO + PE (1.0 mg/kg body wt). Animals were killed at 01:00 hr and pineals were collected for 5′-D activity determinations. Results are the mean ± SE of eight animals per group. a, $P < 0.001$ versus SAL.

ISO, a β-adrenergic receptor agonist only, in stimulating pineal 5′-D activity. Consequently, in this study ISO was injected in combination with PE, an α-adrenergic agonist. As shown in Figure 2, PE injections did not statistically significantly potentiate the effect of ISO alone, suggesting that β-adrenergic receptors are mainly involved in rat pineal 5′-D activation under *in vivo* conditions.

Experiment 3. An additional experiment was performed to study the role of α-adrenergic receptors in the nocturnal increase of rat pineal 5′-D activity. In this experiment, hypothyroid animals were permitted to enter darkness at 20:00 hr; at the time of darkness onset, the rats were injected with either saline, PRAZ, an α-adrenergic receptor blocker, or PROP, a β-adrenergic receptor blocker. As shown in Figure 3, PRAZ did not modify the nocturnal increase in rat pineal 5′-D activity. However, propranolol highly significantly depressed the nocturnal activity of the T_3-forming enzyme.

Discussion

The regulation of type II thyroxine 5′-D activity has been studied in a number of tissues and species. Circulating levels of thyroid hormone is both the most important and common regulatory factor described for the 5′-deiodinase isoenzyme. The activity of this isoenzyme depends on circulating T_4 levels with 5′-D activity being stimulated by depressed T_4 titers (11, 12, 14). This was also found to be the case in the present series of studies (Experiment 1). 5′-D Activity is believed to have an important role in maintaining the intracellular

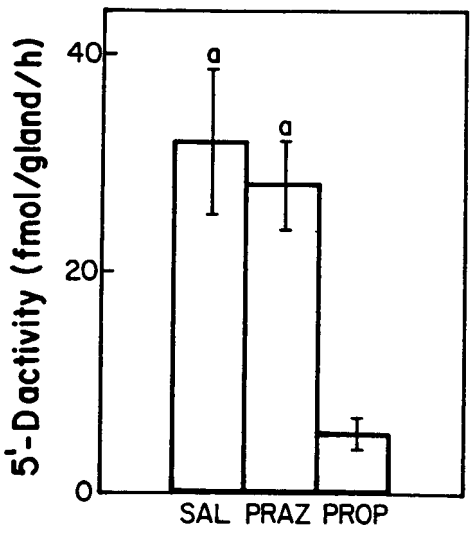

Figure 3. Effect of either PRAZ or PROP on the nocturnal increase of pineal 5′-D activity in euthyroid rats. Animals were permitted to enter darkness at 20:00 hr and were subcutaneously injected (20:00 hr) with a single dose of either saline (SAL), PRAZ (20 mg/kg body wt), or PROP (20 mg/kg body wt). Animals were killed at 01:00 hr and pineals were collected for 5′-D activity determinations. Results are the mean ± SE of eight animals per group. a, $P < 0.001$ versus PROP.

levels of T_3 serving as a defense against thyroid hormone deficiency.

Besides the thyroid status, the noradrenergic system also regulates type II 5′-D activity. Thus, in rat pineal, noradrenergic input to the gland is generally believed to be responsible for the nocturnal increase in not only pineal *N*-acetyltransferase activity and melatonin production but also 5′-D activity (2, 15, 16, 18). In the gland, stimulation of N-acetyltransferase activity is seemingly always accompanied by an increase in 5′-D. The importance of β-adrenergic receptors in the induction of rat pineal 5′-D was shown earlier *in vivo* and *in vitro* studies were ISO, a β-adrenergic agonist, was able to stimulate 5′-D activity (17, 19–22). The activation of 5′-D activity by isoproterenol is not an exclusive feature of the pineal gland. In addition to the rat pineal gland, 5′-D activity in the Syrian hamster Harderian gland is also stimulated by isoproterenol (25) and inhibited by the β-receptor blocker propranolol (26). In contrast, in brown adipose tissue, the noradrenergic input increases type II 5′-D activity by acting via a α-adrenergic receptor mechanism (13).

In the present report we show, for the first time, the *in vivo* activation of rat pineal 5′-D activity by injecting NE, the physiologic neurotransmitter released from the sympathetic nerve endings within the gland. In a previous report, NE, when injected without a reuptake blocker, was incapable of stimulating rat pineal 5′-D activity (22). In the current study we administered NE in conjunction with DMI, a drug which inhibits NE reuptake by the postganglionic sympathetic terminals. Under these conditions NE stimulated rat pineal 5′-D activity (Fig. 1). Moreover, the response to NE was greater in pineals collected from hypothyroid animals confirming that, during hypothyroidism, the pineal gland is supersensitive to adrenergic agonists probably due to the presence of larger quantities of mRNA for the synthesis of the 5′-D enzyme (20).

We had previously shown in vivo that PE, an α-adrenergic agonist, by itself had no effect on rat pineal 5′-D activity (17). In Experiment 2, both ISO and PE were injected into some rats. Under these circumstances PE also did not statistically potentiate the effect of ISO on pineal 5′-D activity. In Experiment 3, the nocturnal increase in pineal 5′-D activity was completely abolished by PROP (a β-receptor blocker) while PRAZ, an α-adrenergic receptor blocker, had no effect. These results indicate that the role of α-receptors in mediating rat pineal 5′-D activity, if it does exist, is minor in contrast to the role of the β-adrenergic receptors.

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