Nocturnal Increase of Type II Thyroxine 5′-Deiodinase Activity in the Syrian Hamster Harderian Gland is Abolished by Light Exposure and Induced by Isoproterenol[1] (42848)

J. M. GUERRERO, A. GONZALEZ-BRITO, C. SANTANA, AND R. J. REITER
Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, Texas 78284

Abstract. **The presence of type II 5′-deiodinase activity in the Syrian hamster Harderian gland was investigated. This enzyme exhibited an increase of its activity after animals entered the normal dark phase, with maximal activity occurring at 04.00 hr (8 hr after lights off). The nocturnal increase was prevented by maintaining the animals in light during the night. Isoproterenol subcutaneously injected every 2 hr (1.0 mg/kg body wt) from 20.00 hr through 0.400 hr to animals exposed to light during the normal dark period mimicked the effect of darkness, i.e., with this treatment an increase in 5′-deiodinase activity with maximal peak values at 02.00 hr was observed. The results show that 5′-deiodinase activity in the Syrian hamster Harderian gland exhibits a nyctohemeral profile dependent on β-adrenergic activation of the gland.** [P.S.E.B.M. 1989, Vol 190]

Type II 5′-deiodinase (5′-D) activity is present in the rat pineal gland (1–3). This enzyme converts the relatively inactive thyroid hormone, thyroxine (T_4), into its highly active metabolite, triiodothyronine (T_3). The most important regulatory mechanism for this enzyme is the thyroid status, exhibiting an important increase in its activity during hypothyroidism (2, 4). In the rat gland, besides the thyroid status, 5′-D activity is also regulated by the light:dark cycle with a progressive rise in 5′-D activity after the onset of the darkness with peak values being reached 5–6 hr later (2–4). The nocturnal rise seems to be dependent on the sympathetic noradrenergic input since either continuous light exposure or superior cervical ganglionectomy prevents it (3, 5). Additionally, isoproterenol, a β-adrenergic agonist, also increases 5′-D activity while, propranolol, a β-blocker, inhibits it (2, 6, 7).

In the rat Harderian gland, where rhythmic patterns in melatonin similar to those seen in the pineal gland, although with different phasing, have been described (8, 9), 5′-D activity also is present (10). As occurs in pineal gland, the activity of the T_4-deiodinating enzyme in Harderian gland increases with hypothyroidism and exhibits a marked nyctohemeral profile reaching maximal values late in the dark period (10). No data concerning the regulatory mechanisms of the nyctohemeral increase in 5′-D activity in rat Harderian gland are available. The present work describes Harderian gland 5′-D activity in another rodent, the Syrian hamster. In this species, as in the rat, rhythms in the melatonin content (11) as well as in the enzymes involved in the conversion of serotonin to melatonin, i.e., *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase (12), have been observed in the Harderian gland. We report herein that in the Syrian hamster Harderian gland, 5′-D activity is also present, exhibiting a nocturnal increase that is prevented by maintaining animals under light at night. Moreover, the nocturnal increase in 5′-D activity in the hamster Harderian gland is mimicked by injecting isoproterenol, a β-adrenergic agonist, to animals maintained under light at night.

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Materials and Methods

Male Syrian hamsters, weighing 80–100 g at the time of the study, were purchased from Sasco (Omaha, NE) and allowed to acclimate to the animal facilities. Animals received food and water *ad libitum* and were exposed to an automatically regulated light:dark cycle of 14:10; the lights were turned off daily from 2000 hr through 0600 hr. On the night of the experiments, hamsters were killed by decapitation at the indicated times and Harderian glands were collected, frozen in solid CO_2, and stored at −70°C until assayed for 5′-D activity.

The measurement of 5′-D 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′. Other deiodinating activities, i.e., conversion of T_4 to rT_3, would release only nonradioactive iodide (2). Briefly, Harderian glands were homogenized with a Polytron (Kinematica, Lucerne, Switzerland) in 1 ml of 0.5 *M* phosphate buffer and 1 m*M* EDTA (pH 7.0), and then were centrifuged for 20 min at 500*g*; the infranatant of the superficial upper lipidic layer was used for the determination of 5′-D activity. Incubations were carried out on 100 μl of the infranatant in the presence of 40 n*M* D,L-dithiothreitol and 2 n*M* [3′,5′-^{125}I]T_4 (200 μl of the final volume). The tracer concentration was similar to the K_m value described for 5′-D activity in rat Harderian gland (10). The 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 800 μl of 10% trichloroacetic acid. The samples were centrifuged for 10 min at 3000 rpm, and 500 μl of the supernatant were decanted onto a 0.5-ml column packed with Dowex-50W ion exchange resin and eluted with 1 ml of 10% glacial acetic acid (13). 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 greater than 95%. Specific enzymatic release of ^{125}I was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added, and referred to as femtomoles of iodine released/mg protein/hr. Proteins were measured by the method described by Lowry *et al.* (14), using bovine serum albumin as the standard. Results are expressed as means ± standard errors. Data were statistically analyzed using an analysis of variance followed by a Student-Newman-Keuls multiple range test.

All reagents were of analytical grade and obtained from commercial sources. T_4, T_3, D,L-dithiothreitol and (−)-isoproterenol 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 by Nakamura *et al.* (15), 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.

Two experiments were designed to study the 5′-D activity regulation in the Syrian hamster Harderian gland.

Experiment 1. Fifty-six hamsters (seven groups of eight each) were used in this study. On the day of the experiment, a group of animals was killed at each of the following times, 10.00, 20.00, 00.00, 04.00, 06.00, or 10.00 hr, to study the 24-hr profile of 5′-D activity in Harderian gland. Animals killed during the normal dark phase period (from 20.00 hr to 06.00 hr) were decapitated under a dim red light. An additional group was maintained under light at night (50 $\mu W/cm^2$) instead of entering the normal dark phase at 20.00 hr and then killed at 04.00 hr.

Experiment 2. Forty hamsters (five groups of eight each) were used in this experiment. On the night of the experiment, all animals were maintained under light (50 $\mu W/cm^2$) from 20.00 hr until they were killed. At the same time, groups of animals were repeatedly injected subcutaneously with isoproterenol (1.0 mg/kg body wt) at 20.00, 22.00, 00.00, 02.00, and 04.00 hr and groups of eight animals each were killed at 20.00, 00.00, 02.00, 04.00, or 06.00 hr.

Results

Experiment 1. The 24-hr profile of 5′-D activity in the Syrian hamster Harderian gland showed a nyctohemeral increase (Fig. 1). After the onset of darkness, enzyme activity increased progressively until a maximal peak value at 04.00 hr. Thus, basal values of 1.23 ± 0.31 fmol/mg protein/hr at 20.00 hr were increased during the night to 4.77 ± 1.07 fmol/mg protein/hr by 04.00 hr (4-fold above basal levels). However, animals maintained under light at night did not exhibit an increase in the Harderian gland 5′-D activity (1.34 ± 0.27 fmol/mg protein/hr) when killed at the time maximal 5′-D activity was reached in darkness (04.00 hr).

Experiment 2. The purpose of this study was to investigate whether we could reproduce the nocturnal pattern of Harderian gland 5′-D activity in animals maintained under light conditions at night by giving repeated injections of isoproterenol, a β-adrenergic agonist. As shown in Figure 2, isoproterenol elicited a progressive increase in Harderian gland 5′-D activity, reaching a maximal peak value at 02.00 hr (11.32 ± 1.97 fmol/mg protein/hr). The response of Harderian gland 5′-D activity to isoproterenol had a maximal peak value 2 hr before that found in animals maintained in darkness (cf., Figs. 1 and 2).

Discussion

In rodents, the Harderian gland is a large tubuloalveolar gland which is located in the posteromedial aspect of the orbital cavity (16). Initially, these glands

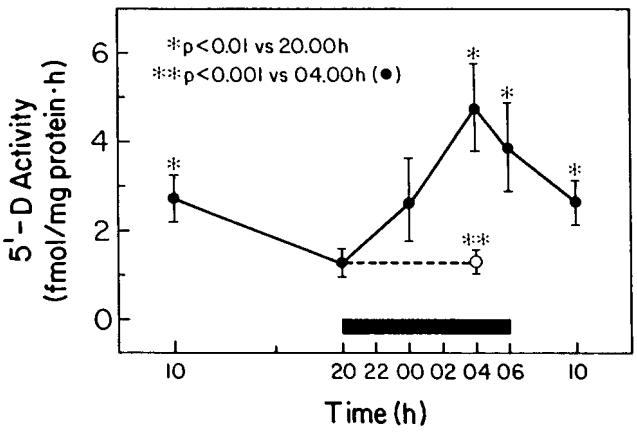

Figure 1. Nyctohemeral profile of 5′-D activity in Syrian hamster Harderian glands (●). One group of animals (○) was maintained under light rather than entering darkness at 20.00 hr. Animals were killed at the indicated times and Harderian glands were collected for 5′-D activity determinations. Results are mean ± SEM of eight animals/group.

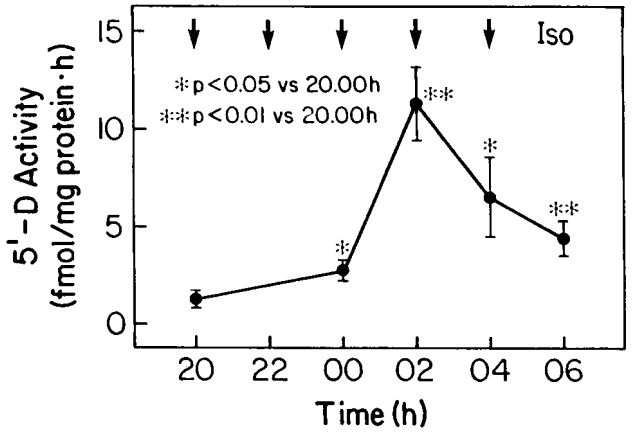

Figure 2. Effect of isoproterenol on Harderian gland 5′-D activity of Syrian hamsters. Rather than entering darkness at 20.00 hr, hamsters were maintained under light and were repeatedly injected subcutaneously with isoproterenol (Iso) (1.0 mg/kg body wt) at the indicated times (arrows). Groups of animals were killed at either 20.00, 00.00, 02.00, 04.00, or 06.00 hr and Harderian glands were collected for 5′-D activity determinations. Results are mean ± SEM of eight animals/group.

were presumed to function in lubricating the cornea and the nicitating membrane (17). Recently, several other functions have been proposed for the rodent Harderian gland including the transfer of photic information to the pineal gland (18), modulation of reproductive function (19, 20), production of pheromones affecting aggression and sexual behavior (21), and regulation of the body temperature (22). In the latter context, the presence of the enzyme Type II thyroxine 5′-deiodinase, which converts the most abundant thyroid hormone, T_4, to the active metabolite, T_3, suggesting that this organ may have a high metabolic activity.

5′-D activity has been described in the rat Harderian gland (10). The role of T_3, produced by 5′-D activity, in this gland is unknown but the enzyme exhibits a nocturnal increase similar to that described for this enzyme in rat pineal gland (1). Moreover, the rat Harderian gland also contains melatonin (9), the chief hormonal product of the pineal gland. The Syrian hamster Harderian gland also contains 5′-D activity, as shown herein, as well as melatonin and the enzymes involved in its synthesis, *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase (11, 12). The activity of the T_4-metabolizing enzyme exhibits a nyctohemeral rise with the increase occurring after the onset of the darkness and reaching a maximal peak late at night (04.00 hr). The rhythm is similar to that previously described for rat Harderian gland (10). We also report here that the nocturnal increase in Harderian 5′-D activity is prevented if hamsters are exposed to light at night. The results suggest that the lighting environment regulates the activity of the enzyme in the Syrian hamster gland as occurs with 5′-D activity in rat pineal gland (10). 5′-D activity has not been studied in the hamster pineal gland.

Clearly, the nocturnal increase in 5′-D activity in the Harderian gland of light-exposed hamsters can be induced by injecting isoproterenol, a β-adrenergic agonist. Isoproterenol was found to be a potent activator of Harderian gland 5′-D activity, and when the drug was injected at 2-hr intervals it was able to produce an increase in activity similar to that seen during the normal dark phase. The results suggest that the β-adrenergic innervation of the Harderian gland may mediate the nyctohemeral rise in 5′-D activity in the Harderian gland. However, since the noradrenergic fibers innervating the Harderian gland end primarily on the blood vessels within the organ (23) (rather than on the secretory cells), it would seem that 5′-D activity in the Harderian gland is regulated differently than in the pineal gland where the postganglionic sympathetic nerves terminate directly on the secretory cells of the gland.

There is at least one other explanation for the effect of isoproterenol on 5′-D activity in the Harderian gland. Thus, the β-receptor agonist may have stimulated an intermediate factor that subsequently activated 5′-D activity. Puig-Domingo *et al.* (24) recently showed that 5′-D activity in brown adipose tissue is stimulated by melatonin injections. Since isoproterenol is a well known stimulator of melatonin production (8), the rise in melatonin following β-receptor agonist administration may have induced the rise in 5′-D activity in the Harderian gland. This is consistent with the suppression of 5′-D activity at night in light-exposed hamsters; light also is known to suppress melatonin production in the hamster pineal gland (25). Further studies are required to elucidate whether the noradrenergic input of the hamster Harderian gland regulates 5′-D activity either directly or indirectly through other messengers.

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