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Continuous light exposure modifies the nocturnal increase in rat thymus type II thyroxine 5′-deiodinase

M. Soutto, P. Molinero* and J. M. Guerrero

Department of Medical Biochemistry and Molecular Biology, School of Medicine, University of Sevilla and Virgen Macarena Hospital, Avda Sánchez Pizjuán 4, E-41009 Sevilla (Spain), Fax +34 54907048

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Abstract. In the present study we show that thymus type II thyroxine deiodinase activity exhibits a nyctohemeral profile, with basal values during the day and high values at night. This rhythmic character is dependent on neuroadrenergic input since exposure to continuous light at night completely abolished the nocturnal rise of the enzyme activity. However, treatment with isoproterenol under light exposure at night restored it.

Key words. Thymus; thyroxine; deiodinase; continuous light; isoproterenol.

Thyroxine type II 5′-deiodinase (5′D-II) is responsible for producing most of the intracellular T_3, which is the most active form of thyroid hormone. The enzyme is found predominantly in brain [1], anterior pituitary [2], brown adipose tissue [3], epidermal keratinocytes [4], the Harderian and pineal glands [5, 6], and thymus [7]. The most important regulatory factor for this enzyme is the thyroid status; there is an increase in enzyme activity during hypothyroidism and a marked inhibition in the presence of T_4 [8]. In the pineal and Harderian glands, besides the thyroid status 5′D-II is also regulated by the light:dark cycle. Enzyme activity shows a progressive rise after the onset of the dark period and reaches a peak value 5–6 h later. This nocturnal increase is dependent on sympathetic noradrenergic input, since either continuous light exposure or superior cervical ganglionectomy prevents it [9, 10]. In addition, isoproterenol, a β-adrenergic agonist, also activates 5′D-II while propranolol, a β-adrenergic blocker, inhibits it [11, 12]. In thymus, 5′D-II activity is regulated by catecholamines as well as by the thyroid status, since β-adrenergic agonists stimulate the enzyme [13].

In the present study we show for the first time that continuous light exposure inhibits the nocturnal increase of 5′D-II activity in the rat thymus.

Materials and methods

Wistar rats of both sexes weighing approximately 120 g were used. No differences between male and female rats in terms of the enzyme activity were found (1.75 ± 0.22 vs 1.82 ± 0.16 femtomoles of ^{125}I released per mg protein/h at 1400 h). Animals received food and water *ad libitum* and were exposed to an automatically regulated light:dark (LD) cycle of 14:10; the lights were turned off daily from 2000 to 0600 h. On the day of the experiment, animals were killed by decapitation at the times indicated. Thymuses were quickly collected, frozen on solid CO_2, and stored at -70 °C for 5′D-II determinations.

The measurement of deiodinase activity was based on the release of radioiodine from [3′,5′-^{125}I]T_4 [6]. This activity is specific for 5′D-II, since the substrate used contains ^{125}I in position 5′. Other deiodinating activities, e.g. conversion of T_4 to rT_3, would release only non-radioactive iodine. Before the assay, thymuses were homogenized in cold 0.05 M phosphate buffer, pH 6.8 (100 mg/ml). Then, 100 µl of the homogenate (corre-

* Corresponding author.

sponding to 6 mg protein/ml) were incubated in the presence of 40 mM DTT and 2 nM [3′,5′-^{125}I]T_4 as substrate (200 µl final volume). The substrate concentration was similar to the K_m value described for 5′D-II activity in rat thymus [7]. 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 homogenate. The reaction was terminated by the addition of 100 µl cold 2% BSA and 750 µl 10% trichloroacetic acid. The samples were centrifuged for 10 min at 3,000 rpm and the supernatant was decanted onto a 0.5 ml column packed with Dowex-50W ion exchange resin, and eluted with 2 ml 10% glacial acetic acid. Radioactivity in the eluate, corresponding to the ^{125}I released, was counted in a gamma counter as an index of 5′D-II activity. The recovery of ^{125}I in this process was greater than 95%. Specific enzymatic activity (approximately 3% of total counts) was determined by subtracting the control value, which usually amounted to less than 1% of the total radioactivity added. 5′D-II activity is referred to as femtomoles of ^{125}I released per mg protein/h. Amounts of proteins were measured by the method described by Bradford [14]. Results are expressed as means ± standard errors (SEM). Significant differences between groups were determined by ANOVA followed by Newman-Keuls t-tests.

All reagents were of analytical grade and obtained from commercial sources. T_3, D,L-dithiothreitol (DTT), (−)-isoproterenol (ISO), and Dowex-50W were purchased from Sigma (St. Louis, MO); Na ^{125}I was purchased from Amersham (Amersham, UK). ^{125}I was bound to T_3 using the chloramine-T method, as described by Nakamura et al. [15]. [3′,5′-^{125}I]T_4 was purified through a 3 ml Sephadex LH-20 column to contain less than 2% free iodine according to the purified tracer, and was used immediately for 5′D-II analyses.

Results

Rat thymus 5′D-II exhibited changes during the light:dark cycle (fig. 1). The enzyme activity showed low values during the day, increasing its activity at the beginning of the dark period and reaching maximal values at 0300 h (3-fold above basal level).

In the second experiment, four groups of rats were sacrificed under different conditions. One group was allowed to enter into the normal dark period at 2000 h, and these rats were killed at 0200 h. Another group was maintained under light conditions instead of entering into darkness, and then was also killed at 0200 h. The last group was also maintained under light conditions at night, but animals were given subcutaneous injections of isoproterenol (1 mg/kg bw). Isoproterenol injections were repeated at 2000 h, 2300 h and 0100 h, and animals were killed one hour after the last injection (0200 h). The control group was killed during the day at 1400 h. As shown in figure 2, thymus 5′D-II activity increased when rats were maintained in darkness, as occurred in the first experimental group. On the other

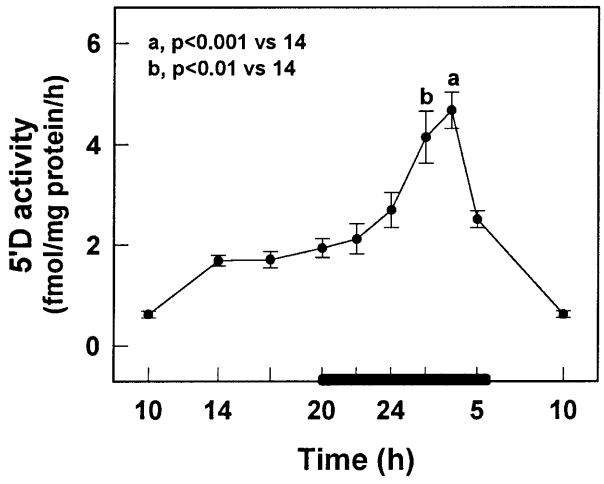

Figure 1. Twenty-four hour rhythm of 5′D-II activity in rat thymus. On the day of experiment, animals were maintained under a normal daily 14:10 light:dark (LD) cycle. Animals were killed at the times indicated, and thymuses were collected for estimation of enzyme activity. Values are expressed as means ± SEM of 10 animals.

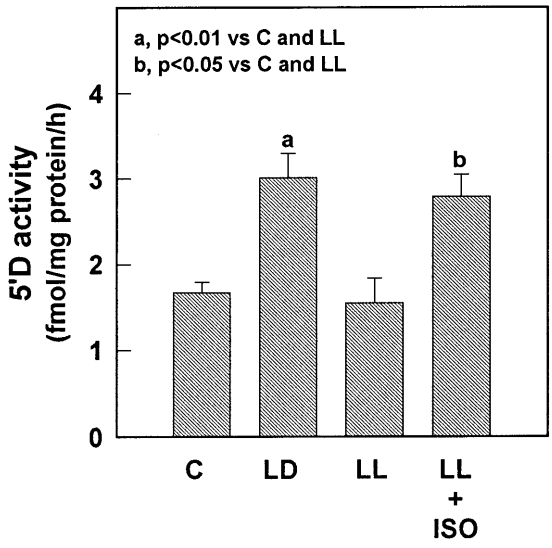

Figure 2. Effect of darkness (LD), light exposure at night (LL), and isoproterenol (LL + ISO) on thymus 5′D-II activity. Groups of animals were maintained under the normal dark period, under light exposure at night, or under light exposure at night plus isoproterenol injections. Animals were killed at 0200 h. Control group (C) was killed at 1300 h. Each value is the mean ± SEM of 10 animals.

hand, light exposure at night abolished the nocturnal increase in the enzyme. However, injecting isoproterenol into animals kept under light conditions at night restored 5′D-II activity to the levels of animals kept in darkness.

Discussion

These results demonstrate a circadian periodicity of 5′D-II activity in the rat thymus that is synchronized to the light:dark cycle. The rhythmic character of type II 5′D activity has been clearly established in some tissues, such as the pineal [9] and Harderian [10] glands, indicating that the circadian rhythmicity of this enzyme activity is more common than we used to think, particularly in tissues where the enzyme is regulated by noradrenergic input in addition to the thyroid status. In the rat thymus, 5′D-II activity was first described by Molinero et al. (1995). This deiodinating activity was regulated by thyroid status [7] and the adrenergic mechanisms [13]. In ths present work, we show that thymus 5′D-II activity also exhibits a nyctohemeral profile. This rhythmicity, as in other tissues, seems to be dependent on sympathetic innervation since light exposure at night completely prevents it. However, treatment with isoproterenol, a β-adrenergic agonist, under light exposure at night restored 5′D activity to the dark period values.

Numerous studies have also reported circadian periodicity in components of the immune system [16] and in immune function [17]. Moreover, a circadian rhythm has been demonstrated in thymosin-α_1, a thymic peptide that influences lymphocyte differentiation and activity [18]. Because the thymus is known to be an organ under the influence of other endocrine tissues [19], it is possible that the 5′D-II activity rhythm is a consequence of some other hormonal rhythms. It has been postulated that the pineal gland has a regulatory function over the thymus, but a direct role of melatonin in this process remains to be demonstrated [20].

In conclusion, our results show nyctohemeral fluctuations of 5′D-II activity in the rat thymus. This enzyme activity is dependent on sympathetic noradrenergic input, since continuous light at night inhibited the nocturnal rise, and isoproterenol restored it. These results contribute to our understanding of the regulatory mechanism of the type II 5′D in thymus. The question of whether such modulation is relevant to the peripheral efficiency of the immune system remains to be answered.

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