Thyroid hormone maintains normal circadian rhythm
of blood corticosterone levels in the rat
by restoring the release and
synthesis of ACTH after thyroidectomy

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Abstract. The role of thyroid hormone in maintenance of the circadian adrenocortical rhythm was studied in the rat. The amplitude of the circadian rhythm of blood corticosterone levels gradually decreased with time after thyroidectomy, and by 5 weeks the rhythm disappeared. However, the circadian peak elevation constantly occurred just before the onset of the dark period until 4 weeks. Three weeks after thyroidectomy, daily overall treatment with thyroxine (T₄) or 3,5,3'-triiodothyronine (T₃) completely restored the amplitude of the circadian adrenocortical rhythm to the previous level within 2 weeks. Thyroidectomy did not affect the circadian rhythm in water intake. However, thyroidectomy resulted in a loss of significant difference of plasma adrenocorticotrophin (ACTH) levels between the morning and the evening, by decreasing the evening levels. Similarly, pituitary ACTH content was decreased by thyroidectomy. Replacement of T₄ completely restored the decreased ACTH levels to the previous ones. These results suggest that thyroid hormone plays an important role in maintenance of the normal amplitude in circadian adrenocortical rhythm in the rat, by affecting ACTH synthesis.

There have been some observations which seem to indicate that factors other than ACTH are involved in control of adrenocortical rhythm in the rat. The rhythm in plasma ACTH has a much smaller amplitude than that of plasma corticosterone, and the difference of ACTH levels between the morning and the evening often does not reach statistical significance (Engeland et al. 1977; Dallman et al. 1978; Wilkinson et al. 1979). The rhythm of adrenal responsiveness to ACTH was found to persist in rats pre-treated with dexamethasone (Dallman et al. 1978; Kaneko et al. 1980). The injection of fixed amounts of ACTH maintained the corticosterone rhythm in hypophysectomized rats (Ottenweller et al. 1978).

Recently, it has been shown that the thyroid plays an important role in circadian adrenocortical rhythm (Meier 1976; Ottenweller & Hedge 1981). Ottenweller & Hedge (1981) reported that thyroidectomy resulted in disruption of circadian adrenocortical rhythm. However, they failed to demonstrate a complete loss of rhythm, since the highest and the lowest levels appeared at light-dark and dark-light transition, respectively. In addition, thyroid hormone causes a change in the free-running period of the circadian rhythm, by direct action on the circadian oscillator (Beasely & Nelson 1982).

In the present study, we confirmed the effect of thyroidectomy on the circadian adrenocortical rhythm in rats, and attempted to elucidate the mechanism of the abolition of adrenocortical rhythm by thyroidectomy.

Materials and Methods

Adult female Wistar albino rats were housed 4 per plastic cage with a controlled thermal environment (22 ± 1°C) and allowed to feed ad libitum. The light schedule was maintained with 12 h of light (07.00–19.00 h) and 12 h of dark (19.00–07.00 h).
Thyroidectomy and sham operation were performed under ether anaesthesia. After surgery, the rats were supplied with 1% calcium lactate solution, rather than plain water, for drinking. Blood samples were taken from individual rats at 4 h intervals for 24 h, once a week, for 5 consecutive weeks by the tail tip incision method (Takahashi et al. 1979). In the 5th week, water intake was determined in some individual rats, at 4 h intervals for 48 h. Three weeks after thyroidectomy, some rats were provided with drinking water containing T4 (1.5 µg/ml) or T3 (0.1 µg) in 1% calcium lactate solution for 2 weeks. During this period of replacement with T4 or T3, blood samples were obtained from individual rats at 4 h intervals for 24 h.

To determine plasma and pituitary ACTH levels, thyroidectomized, sham operated or T4 replaced rats were caged singly. Two or 3 weeks after thyroidectomy and 2 weeks after replacement of T4, rats were killed by decapitation at 09.00 h (a.m. group) and 17.00 h (p.m. group). Blood samples were collected in tubes containing EDTA (1 mg/ml) and trasylo (250 IU/ml). The pituitary was obtained by decapitation at 17.00 h, 2 or 3 weeks after thyroidectomy, and was homogenized with 0.5 ml 0.005 N HCl, 0.9% NaCl solution. After centrifugation at 10,000 r.p.m. for 10 min at 4°C, 100 µl supernatant fluid was collected, and 5 µl trasylo added.

Thirty rats were thyroidectomized and kept with 1% calcium lactate solution for 3 weeks. The adrenals were obtained. The capsule and medulla were removed and the cortex cells were dispersed with 0.4% collagenase solution (Murakami et al. 1982). Some cells were incubated with 0.5 mM DME medium containing 5% foetal calf serum and 20% horse serum in 1.5 mm Nuclon plastic dishes at a concentration of 1 x 10^5 cells/dish under a humidified atmosphere of 7% CO2, 93% air at 37°C. The other cells were incubated with DME medium containing 20% horse serum, 5% foetal calf serum and T3 (various doses). Each medium incubated was changed every 2 days. On the 9th day of monolayer culture, cells were incubated at 37°C for 30 min with Hepes buffer (pH 7.2). Thereafter, cells were incubated at 37°C for 15, 30, 60 or 120 min with buffer containing T3 and/or 0.05 mIU ACTH. After medium was removed at the termination of incubation, corticosterone concentrations in the medium were determined.

Blood corticosterone levels were determined by the method described in a previous paper (Takahashi et al. 1979). Briefly, 1 ml ethanol was added to the test tube containing the blood sample (20 µl). After agitation, followed by centrifugation, 0.5 ml supernatant was transferred to a test tube and evaporated to dryness by air. To each tube, 1 ml corticosterone-binding globulin-isotope solution was added. The corticosterone-binding globulin-isotope solution consisted of 0.3 ml human plasma, 4 µCi [1,2,6,7-3H]corticosterone (New England Nuclear Corp.), and distilled water to a final volume of 100 ml. After agitation, the tubes were placed in a 40°C bath for 10 min with constant agitation and cooled in an ice-water bath for 10 min. Florisil (250 mg) was then added to each tube to separate unbound [3H]corticosterone. The tubes were agitated vigorously for 3 min in the bath and kept there for another 5 min. After Florisil settled, 0.5 ml supernatant was transferred into vials containing 5 ml scintillator solution and radioactivity was counted. Plasma and pituitary ACTH levels were determined by the ACTH radioimmunoassay kit (Midori Judji Corp.). Comparison of bioassayable ACTH and immuneassayable ACTH in the same pituitary sample yielded B/I ratio of 0.97 ± 0.08.

![Figure 1](image-url)

**Fig. 1.**

Twenty-four hour patterns of blood corticosterone levels in the thyroidectomized or sham operated rats determined over a 3 week period after operation. The patterns of 6 individual thyroidectomized rats (T1-T6) and mean levels are shown in the left part of the figure, and the right figure illustrates the mean ± SEM of 12 sham operated rats. For this and subsequent figures, the shaded areas illustrate the dark period. Asterisks indicate significant difference between time of day (* P < 0.01, ** P < 0.05).
were (\(n = 6\)). The minimum detectable level was 1.5 pg/tube. The intra-assay and inter-assay coefficients of variation were 4.9\% (\(n = 6\)) and 7.9\% (\(n = 8\)), respectively.

Student's \(t\)-test was employed for statistical comparisons between experimental groups, while one-way analysis of variance was used to analyze the circadian variation of blood corticosterone levels obtained by serial sampling.

Results

Twenty-four hour patterns of blood corticosterone levels in 6 individual thyroidectomized rats and mean levels are shown in Fig. 1. The blood corticosterone levels of sham operated rats showed a clear circadian rhythm with a peak at the onset of the dark period. The highest level was 34.2 ± 4.2 µg/100 ml. Although the thyroidectomized rats also showed a significant circadian rhythm of mean levels of blood corticosterone for 3 weeks after operation, the amplitude gradually decreased with time after thyroidectomy. Three weeks after thyroidectomy, very low levels of corticosterone were maintained throughout the 24 h period in about half of the rats. Five weeks after thyroidectomy the 24 h patterns of the mean level were flat. In contrast, the circadian rhythm in water intake persisted until the 5th week after thyroidectomy in all rats (Fig. 2). The overall treatment with \(T_4\) or \(T_3\) restored the amplitude of circadian adrenocortical rhythm in thyroidectomized rat to normal levels. In this case, only the peak levels increased, but trough levels constantly remained low (Fig. 3).

Table 1 shows the corticosterone output into cultured medium after addition of \(T_3\) and/or ACTH. A significant increase of corticosterone output into medium was observed 30 and 60 min after addition of ACTH. However, the various doses of \(T_3\) had no stimulative effect on the corticosterone output. In addition, corticosterone output in response to ACTH and \(T_3\) complex was not different from that to ACTH alone. When the adrenocortical cells obtained from long-term thyroidectomized rat were cultured for 9 days with or without \(T_3\), responsiveness to ACTH was indistinguishable between the two groups.

In the sham operated rat, although the plasma ACTH levels had a marked variance, a significant difference was observed between the morning and the evening (Fig. 4). However, there was no significant difference in plasma ACTH levels between the morning and the evening in thyroidectomized rats, since the evening plasma ACTH level was decreased by thyroidectomy. The overall treatment

![Fig. 2.](image)

Forty-eight hour patterns of water intake determined 5 days after thyroidectomy. The two left panels show the results of an individual thyroidectomized rat (T1–T6). Right upper and lower figures illustrate the mean value (± SEM) of sham operated and thyroidectomized rats, respectively.

![Fig. 3.](image)

Effect of replacement of thyroid hormones on the circadian adrenocortical rhythm in thyroidectomized rats. \(T_4\) (left panel) or \(T_3\) (middle panel) mixed with 1\% calcium lactate solution was given as drinking water from 3 to 5 weeks after thyroidectomy. Control rats (right panel) were given 1\% calcium lactate solution only.
Corticosterone output from adrenocortical cells obtained from thyroidectomized rats in monolayer cell culture.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No pre-treatment**</th>
<th>T3 pre-treatment**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>12.8 ± 2.3</td>
<td>25.4 ± 3.2</td>
</tr>
<tr>
<td>ACTH</td>
<td>18.6 ± 2.6</td>
<td>46.8 ± 4.3</td>
</tr>
<tr>
<td>T3 5 × 10^-6</td>
<td>11.8 ± 1.6</td>
<td>26.3 ± 2.5</td>
</tr>
<tr>
<td>5 × 10^-8</td>
<td>11.6 ± 2.0</td>
<td>23.9 ± 2.2</td>
</tr>
<tr>
<td>5 × 10^-10</td>
<td>14.0 ± 4.2</td>
<td>22.5 ± 3.1</td>
</tr>
</tbody>
</table>

ACTH + T3
5 × 10^-6  | 19.4 ± 2.2 | 48.4 ± 5.1 | 90.8 ± 9.0 | 94.3 ± 4.7 | 18.4 ± 3.6 | 46.3 ± 4.7 | 91.4 ± 8.2 | 93.6 ± 5.2 |
5 × 10^-8  | 17.6 ± 5.2 | 44.3 ± 2.6 | 91.1 ± 6.7 | 90.9 ± 6.1 | 20.2 ± 3.2 | 44.6 ± 5.1 | 90.3 ± 5.4 | 94.6 ± 5.5 |
5 × 10^-10 | 18.3 ± 5.7 | 48.2 ± 4.3 | 88.9 ± 4.8 | 92.6 ± 4.4 | 18.6 ± 2.9 | 41.3 ± 4.7 | 88.6 ± 6.2 | 91.5 ± 5.1 |

* The values indicate the mean ± SEM (n = 6, ng/dish) at various times after incubation.
** The cells were pre-incubated with the medium with T3 or without T3 for 9 days.

with T₄ completely restored mean plasma ACTH levels to the control levels. Pituitary ACTH content also decreased significantly 3 weeks after thyroidectomy. However, replacement of T₄ in the thyroidectomized rat restored the pituitary ACTH level to control level (Fig. 5). A significant difference was observed between the T₄-replaced group and the before-replaced group (Tx-T₄ vs Tx 3w).
Thyroidectomy results in decrease of amplitude of circadian adrenocortical rhythm in the rat, and replacement of thyroid hormones completely restores the amplitude to normal level. These results are in agreement with the observation by Ottenweller & Hedge (1981), suggesting that thyroidectomy was probably complete in this study. Ottenweller & Hedge (1981) reported that there was no significant rhythm in plasma corticosterone levels 13 days after thyroidectomy. However, their conclusion seems a little uncertain, because the circadian pattern of plasma corticosterone levels seemed to persist 13 days after thyroidectomy in their paper, with the highest and the lowest levels at light-dark and dark-light transition, respectively. In the present study, although the amplitude was gradually decreased with time after thyroidectomy, a significant rhythm was observed until 4 weeks but not 5 weeks after thyroidectomy. It is likely that they failed to demonstrate complete loss of circadian adrenocortical rhythm by thyroidectomy, because of the short observation period of 7–13 days.

Many investigators have shown that thyroid hormone is required to maintain the normal circadian adrenocortical rhythm (Martin et al. 1963; Meier 1976; Ottenweller & Hedge 1981; Ottenweller & Meier 1982). The amplitude of plasma cortisol rhythm is lower in hypothyroid patients than in euthyroid individuals (Martin et al. 1963). Daily variation in the plasma corticosterone levels is present in thyroidectomized rat implanted ACTH + T₄ pellet (Meier 1976; Ottenweller & Meier 1982). They hypothesized that thyroid hormone may be necessary to maintain the normal function of circadian pace maker. Furthermore, it has been reported that thyroid hormone directly affects the circadian pace maker, since thyroid hormone causes changes in the free-running period of circadian rhythm (Beasley & Nelson 1982). However, the circadian water intake rhythm was not affected by thyroidectomy as demonstrated in Fig. 2. In addition, although the amplitude gradually decreased with time after thyroidectomy, the circadian peak elevation of corticosterone levels constantly occurred just before the onset of the dark period until 4 weeks. This indicates that the circadian adrenocortical rhythm in thyroidectomized rats was linked to light-dark cycle until 4 weeks, since the peak of adrenocortical rhythm always appears just before the dark period in the normal rats (Krieger 1974; Takahashi et al. 1979; Takahashi & Murakami 1982; Murakami et al. 1983; Murakami & Takahashi 1983). Therefore, our observations indicate that thyroidectomy results in the loss of an overt adrenocortical rhythm by decreasing the amplitude but not disrupting the function of circadian pace maker.

It does not seem likely that thyroidectomy decreases the amplitude of circadian adrenocortical rhythm by a direct effect on corticosterone synthesis by the adrenal, since thyroid hormone had no stimulative effect on corticosterone synthesis and response to ACTH in adrenocortical cells obtained from thyroidectomized rat. Furthermore, replacement of thyroid hormone increased the peak level but not the trough level in the thyroidectomized rat (Fig. 3). However, in the cultured experiment, culture medium contained thyroid hormone and corticosteroid of horse or foetal calf serum origin. Therefore, the possibility remains that these thyroid hormones had maximum stimulative effect on corticosterone synthesis or adrenocortical response to ACTH. If this is the case, the effect of thyroid hormone added to the medium may be masked.

Thyroidectomy resulted in decrease in plasma and pituitary ACTH levels. Replacement of T₄ restored the plasma ACTH and corticosterone to normal levels. The decrease in blood corticosterone peak level may be attributable to decrease of plasma ACTH levels. The fact that pituitary ACTH content was decreased by thyroidectomy suggests that thyroid hormone was required to maintain the pituitary ACTH level. In this study, however, it is unknown whether thyroidectomy affected the corticotrophin releasing factor (CRF) or the responsiveness of ACTH to CRF.

In conclusion, thyroid hormones play an important role in maintenance of normal amplitude in the circadian adrenocortical rhythm, by, in part, affecting ACTH release and/or synthesis.

References


Received on January 17th, 1984.