PITUITARY ADRENOCORTICOTROPIC ACTIVITY IN ALTERED THYROID FUNCTION

ATSUO KAWAI

Second Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo

Alteration in thyroid state influenced the rate of metabolism of adrenocortical hormone by rats liver tissue in vitro, with proportional change in adrenal weight (Yates, 1958). These experiments suggest that the thyroid function would determine the rate of ACTH secretion in unstressed animals.

The present study was undertaken to explore the possibility of difference in pituitary-adrenocortical secretion under various stimuli among rats in altered thyroid function.

MATERIALS AND METHODS

Normal female albino rats (Wistar strain) weighing 100~140 g were kept in a thermostatically controlled room (22°C) and were maintained on solid rat diet* and tap water ad libitum. They were separated into 3 groups. Hyperthyroid groups were prepared by feeding of the diet containing 1.5 µg/g (25~30 µg/day) of 3.5.3′L-triiodothyronine (T₃) for 12 days. Hypothyroid groups were prepared by surgical thyro-parathyroidectomy 3 weeks before the study. Untreated rats were used as euthyroid group.

Rats were sacrificed by decapitation with a scissor and arteriovenous blood was collected from neck vessels into heparinized beakers. Plasma corticosterone concentrations were measured in 0.5 ml samples by the method of Guillemin et al. (1959a). Immediately after decapitation the pituitary glands were removed, pooled and extracted by the method of Birmingham et al. (1956). These extracts were assayed for ACTH by the method of in vitro steroidogenetic technique (Saffran and Schally, 1955), except that the incubation medium was estimated for corticosterone by the fluorometric method of Guillemin (1959a). Adrenals were removed, stripped free of adhering tissues. Ascorbic acid concentration was determined by a modification (Guillemin, 1959b) of Roe and Kuether's method (1943).

Procedures for testing the functional integrity of the pituitary-adrenal system:

1) Response to unilateral adrenalectomy

Right adrenalectomy, used as exogenous stress, was performed by lumbar approach under ether anesthesia. To minimize stress-induced depletion of ascorbic acid content of right adrenal gland, the control gland was removed within 2 mins. of anesthesia. Three animals were killed by decapitation at progressively longer intervals (0, 15, 30, 60 and 120 mins.) from the onset of etherization.

2) Effect of SU-4885

An 11β-hydroxylase inhibitor of adrenocortico steroids, SU-4885-ditartrate used as endogeneous stress, was given subcutaneously in aqueous solution at a dose level of 10 mg per 100 g body

Received for publication May 28, 1962.
* Containing iodide approximately 0.1 µg/g.
weight. In order to maintain its concentration in blood, injections of SU-4885 were repeated every hr. Uninjected rats were sacrificed at 1, 2, 3 and 4 hrs. from the 1st injection.

3) Response to exogenous ACTH
An aqueous solution of ACTH, 50 mU/0.1 ml, was administered intraperitoneally at a dose of 50 mU per 100 g body weight. Uninjected rats were sacrificed at once and injected animals at intervals of 15, 30, 60 and 120 mins.

RESULTS

Results in Unstressed Animals
Significant increase in adrenal weight was obtained after administration of T3 for 12 days (Table 1). T3 administration was also associated with a slight increase in pituitary content of ACTH. Thyroidectomized rats, on the other hand, showed a marked depletion in pituitary ACTH to the level of about 60% of the control group. Slight decrease in adrenal weight was observed in the hypothyroid rats, but an increase was observed in pituitary weight over the control group.

Table 1. Adrenal and pituitary weight, pituitary ACTH and plasma corticosterone in unstressed rats

<table>
<thead>
<tr>
<th></th>
<th>Adrenal weight † (mg/100 g)</th>
<th>Pituitary weight † (mg/100 g)</th>
<th>Pituitary ACTH†† (mU/pituitary)</th>
<th>Plasma corticosterone† (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>21.4±2.65</td>
<td>4.33±0.61</td>
<td>266±18</td>
<td>15.5±2.1</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>30.3±3.89**</td>
<td>4.33±0.41</td>
<td>279±23</td>
<td>14.3±1.8</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>19.1±4.58</td>
<td>5.10±0.66**</td>
<td>178±25*</td>
<td>16.0±2.3</td>
</tr>
</tbody>
</table>

† Mean±standard error
†† Antilog M±2.303 sm (Antilog M)
* P<0.05 (Compared to euthyroid animals)
** P<0.01 (Compared to euthyroid animals)

The plasma corticosterone concentration of animals in either hyper- or hypothyroid state did not differ significantly from those of the control group.

Response to Unilateral Adrenalectomy

1) Plasma corticosterone level
Following the application of surgical procedure, euthyroid rats showed a prompt rise in plasma corticosterone concentration to a peak of 62.5±4.3 µg/100 ml at 30 mins. (Fig. 1). Hyperthyroid rats also demonstrated a peak plasma steroid level at 30 mins. post-stress. However, the plasma concentration attained was strikingly higher, (P<0.01), 84.8±5.3 µg/100 ml. The value 52.4±3.3 µg/100 ml observed at 120 mins. was significantly higher than the euthyroid group (P<0.01).

Hypothyroid rats, on the contrary, showed a rather slower rise in plasma corticosterone concentration to a peak of 53.4±4.1 µg/100 ml at 30 mins. Plasma level at 120 mins. was significantly lower than the euthyroid group (P<0.01).

2) Adrenal ascorbic acid
Results were expressed as per cent decrease of the ascorbic acid concentration
in left adrenal gland from that in the right adrenal gland (Fig. 2). Following
the surgical stress, rats of each group showed a gradual decrease in adrenal as-
corbic acid concentration and reached the minimum level after 1–2 hrs. Hyper-

Fig. 1. Plasma corticosterone concentrations following unilateral adrenalectomy in rats with altered thyroid functions
Each point represents the mean of 3 observations.

Fig. 2. Ascorbic acid concentration of left adrenal glands following right adrenalectomy
Each point represents the mean of 3 observations, expressed as per cent change from right adrenal levels.

Fig. 3. Pituitary ACTH content following unilateral adrenalectomy in rats of altered thyroid function
Each point represents the mean of 3 pooled pituitary glands.
thyroid rats demonstrated a steeper decline and the maximal depletion, $-52.5\%$ was significantly lower than the euthyroid group ($P<0.05$), while hypothyroid rats showed rather slower slope and the maximal depletion, $-34.7\%$ was significantly higher than the control group ($P<0.01$).

3) Pituitary ACTH

As shown in Figure 3, an abrupt fall of pituitary ACTH content to the level, 31% of the resting value occurred during the first 15 mins. in euthyroid rats. Pituitary ACTH level showed thereafter a gradual rise up to the level about 70% of the control value at 120 mins. post-stress.

Hyperthyroid rats showed a more marked depletion of pituitary ACTH content to the level of 24% of the control value at 15 mins., which was followed by a gradual increase.

Thyroidectomized rats showed a significantly less depletion in pituitary ACTH content at 15 mins. than that of the euthyroid animals ($P<0.05$).

Effect of SU-4885

1) Plasma corticosterone level

With multiple doses of SU-4885 plasma corticosterone concentrations showed a gradual decrease in similar fashion independent of thyroid function and reached maximal decrease approximately 40% of the resting value at 3~4 hrs. after the 1st injection (Fig. 4).

2) Adrenal ascorbic acid

Results were expressed as per cent decrease from the adrenal levels of the control rats (Fig. 5).

Following the injection of the drug, euthyroid rats showed a decrease in adrenal ascorbic acid and reached the maximal depletion, $-44.7\%$ of the control value after 3 hrs., which remained essentially unchanged at the end of 4 hrs. Hyperthyroid rats showed a more rapid decrease in the adrenal ascorbic acid, but the lowest value of $-45.1\%$ after 2 hrs. was significantly different from that of euthyroid rats ($P<0.05$). Hypothyroid rats revealed a slower slope of adrenal ascorbic acid and reached maximal depletion after 3 hrs., which was significantly higher than that of the euthyroid rats ($P<0.01$).

As a control of the stress of repeated injection, to another group of animals was injected saline repeatedly in similar fashion to the injection of SU-4885. One hr. following the 1st injection normal rats showed a little depletion in adrenal ascorbic acid to the level, 7.4% of the control value, which was almost unchanged with repetition of the injection. These findings suggest that the stress of injection per se did not contribute significantly to the adrenal ascorbic acid depletion observed after SU-4885 administration.

3) Pituitary ACTH

Control rats showed a progressively steeper decrease in pituitary ACTH with additional doses of SU-4885 and reached the lowest value 82.5 mU/pituitary at 3 hrs. after the 1st injection. Rats administered with T3 demonstrated more rapid decline in pituitary ACTH and reached the lowest level after 2 hrs., while thyroidectomized rats revealed a rather slower decline and minimum was recorded after 3 hrs. However, the lowest values of the latter 2 groups did not significantly
June 1962

PITUITARY ACTH AND THYROID STATES

Fig. 4. Plasma corticosterone concentrations following hourly repeated subcutaneous injection of 10 mg of SU-4885-ditartrate per 100 g body weight per hr.
Each point represents the mean of 3 observations.

Fig. 5. Adrenal ascorbic acid concentrations following hourly repeated subcutaneous injection of 10 mg of SU-4885-ditartrate per 100 g body weight per hr.
Each point represents the mean of 3 observations, as per cent change from the control adrenal levels.

Fig. 6. Pituitary ACTH content following hourly repeated subcutaneous injection of 10 mg of SU-4885-ditartrate per 100 g body weight per hr.
Each point represents the mean of 3 pooled pituitary glands.

differ from that of control rats.

Response to Exogenous ACTH
Administration of ACTH to euthyroid rats resulted in a peak plasma corti-
costerone level of $67.4 \pm 5.2 \mu g/ml$ at 30 mins. after injection with a gradual decline to the level of $30.1 \pm 4.0 \mu g/100 ml$ at 120 mins. (Fig. 7). Hyperthyroid rats reached a higher peak level of $74.9 \pm 4.4 \mu g/100 ml$ at 15 mins. with rapid return to the resting levels at 120 mins. Hypothyroid rats attained a level of $55.0 \pm 6.1 \mu g/100 ml$ at 30 mins. without essential change at 60 mins. The plasma steroid concentration remained higher than those of other groups at 120 mins.

![Fig. 7. Plasma corticosterone concentrations following intraperitoneal injection of 50 mU of ACTH per 100 g body weight in rats of varied thyroid function](image)

Each point represents the mean of 3 observations.

**DISCUSSION**

Hyperthyroid rats demonstrated markedly higher and more persistently increased plasma levels of corticosterone after surgical stress while hypothyroid rats showed lower and shorter increase in plasma corticosterone level than euthyroid rats.

These data might be explained by: A) a difference in the responsiveness of the adrenal gland to the same amount of ACTH stimulation among rats in altered thyroid states, B) a difference in the amount of ACTH released in response to stress.

Results obtained following administration of ACTH revealed certainly that adrenal responsiveness to tropic hormone is greater in hyperthyroid rats and less in hypothyroid rats. However, this experiment, on the contrary to the stress study, demonstrated more rapid return to the resting plasma level in hyperthyroid group and more prolonged sustain in hypothyroid group, seemingly due to the difference in biological half-life of steroid hormone. From these results the change of adrenal responsiveness to ACTH could hardly be considered as a single contributory factor in altered thyroid states.

An alternative explanation of the difference in adrenal steroid secretion after surgical stress among rats in altered thyroid states is a difference in pituitary responsiveness with respect to ACTH secretion. This hypothesis was tested by
means of determination of adrenal ascorbic acid concentration as well as pituitary ACTH at the same intervals after application of surgical stress.

Hyperthyroid rats revealed more rapid and marked depletion in adrenal ascorbic acid concentration than in the euthyroid rats, suggesting that greater quantity of ACTH was released in response to surgical stress.

Hypothyroid rats, on the other hand, showed much less decline in adrenal ascorbic acid, suggesting that their pituitary glands were less responsive with respect to ACTH secretion under the same stress.

Changes in the level of ACTH in the pituitary gland were selected as a parameter of the secretion rate of ACTH, because ACTH appeared to be mobilized after an acute stress primarily from the pre-existing stores in the pituitary, which was reported previously by Kitay (1959). Hyperthyroid rats revealed more marked depletion in pituitary ACTH content than the hypothyroid rats, suggesting that greater amount of ACTH was released in response to surgical stress than the latter.

As to the in vitro bioassay of ACTH, Roberts (1957) has raised the point of nonspecificity of this procedure, since rat plasma and serum samples are capable of evoking an increased production of corticoids. However, a differential sensitivity of the Sayers estimate of ACTH present (oxycellulose adsorbable) in the plasma and serum when compared with the in vitro estimates can be explained by the adsorption and inactivation rate differences which appear to play a major role (Fortier, 1958).

SU-4885 was used as another trigger of ACTH. In order to obtain a complete inhibition of adrenal steroidogenesis in rats, rather larger dose of SU-4885 than those used in dog (Jenkins et al., 1958) or man (Gold, 1960) was selected to be given by subcutaneous injection repeatedly every hr. Plasma corticosterone showed a similar decrease independent of thyroid state following SU-4885. Nevertheless, from the rate of decrease in pituitary ACTH as well as adrenal ascorbic acid, the pituitary glands of hyperthyroid rats responded more rapidly in response to the decrease in plasma corticosterone levels, while hypothyroid rats were less responsive than control rats.

If the restoration of pituitary ACTH during the experimental period would be negligible, the amount of ACTH released in response to decrease of plasma corticosterone levels may depend upon the pituitary ACTH reserve prior to the administration of SU-4885, since ACTH contents in all groups irrespective of thyroid states reached the same minimum levels after SU-4885 administration. The present findings showing impaired reserve of pituitary ACTH observed in hypothyroidism was consistent with other observation (Gold, 1961). However, the data indicating normal reserve of pituitary ACTH in the case of hyperthyroidism contradict the results obtained by Gold (1961), who reported a subnormal response to intravenous SU-4885 in patients of hyperthyroidism. This difference may be explained by the difference in dose of SU-4885 administered; Gold used, as he commented, rather insufficient dose to achieve complete inhibition of adrenals in patients of hyperthyroidism.

The results of the present work demonstrated that thyroid hormone can play a major part in the pituitary response to stress. Several explanations of the
data might be considered. The first of these is that thyroid hormone would facilitate the release of ACTH from the pituitary gland by accelerating the turnover of corticosteroids. Another hypothesis is that thyroid hormone could act directly on hypothalamus-pituitary system to enable stress to “reset the set point of feed-back control” (Yates et al., 1961) at higher level, provoking of the release of greater amount of ACTH from the pituitary of hyperthyroid rat than the euthyroid animal. Further work should be done to investigate these possibilities.

SUMMARY

Rats were stimulated by different two triggers of ACTH; unilateral adrenalectomy and SU-4885. The rate of ACTH release in response to those stimuli was evaluated by 3 parameters; plasma corticosterone concentration, pituitary ACTH content, and ascorbic acid concentration.

Surgical stress produced higher and more persistently elevated plasma corticosterone concentration in rats administered by triiodothyronine than in the control rats, while the reverse was obvious in thyroidectomized rats. This difference could not be accounted completely by differences in adrenal responsiveness to tropic hormone among them. The findings on the rate of depletion in adrenal ascorbic acid as well as pituitary ACTH clearly indicated that in response to stress the pituitary gland of hyperthyroid rat is more responsive in terms of ACTH release, while that of hypothyroid rat was less responsive than the control rat.

Following SU-4885 plasma corticosterone showed to decrease in similar fashion independent of thyroid function. Nevertheless, hyperthyroid rats released pituitary ACTH more rapidly than euthyroid rats, while hypothyroid rats released it more slowly, in response to the depression of plasma corticosterone concentration.

It is clear from these results that the thyroid hormone is an important factor which controls the capacity of the pituitary gland to release ACTH in response to stress.

ACKNOWLEDGEMENTS

The author wishes to express his hearty thanks to Prof. Toshio Torii, Hokkaido University and Assist. Prof. Yoshihiko Horiuchi for their kind guidances and supports during this investigation.

REFERENCES