Thyroid Hormone Action on ACTH Secretion

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Summary

Thyroid hormone effects on pituitary ACTH have not been well established. Adult male Sprague-Dawley rats were rendered hypo- and hyperthyroid while undergoing treatment with 6-Propylthiouracil (PTU) and L-Thyroxine (T_4). At the time of decapitation, plasma values for T_4 (μg/100 ml) were 3.9 ± 0.4 in the control, 17.3 ± 2.2 in the T_4 and < 2 in the PTU treated group; plasma T_3 and TSH confirmed hyper- and hypothyroidism in the T_4 and PTU treated groups respectively. Plasma immunoassayable ACTH and corticosterone were significantly increased in hyperthyroid and decreased in the PTU treated animals. Pituitaries were removed and incubated in DMEM. After 3 h incubation, ACTH content and secretion to the medium were significantly lower in the PTU group. As expected, pituitary TSH content and secretion were decreased in the T_4 treated animals. These data indicate that thyroid hormones influence pituitary-adrenal function by increasing ACTH secretion and consequently corticosterone production.

Key-Words

ACTH – Corticosterone – Thyroid Hormones – TSH

Introduction

It is generally accepted that adrenocortical function is decreased in primary hypothyroidism (*Linquette, Lefebre, Racapot* and *Cappoen* 1976; *Brien* 1976; *Lessof, Lyne, Maisey* and *Sturge* 1969; *Bigos, Ridgway, Kourides* and *Maloof* 1978) and increased in hyperthyroidism (*Gallagher, Hellman, Finkelstein, Yoshida, Weitzman, Roffwarg* and *Fukushima* 1972). However, the mechanism of these variations has not been well established. In hypothyroidism, reduced cortical function has been attributed to an inappropriate adrenocortical response to ACTH (*Linquette* et al. 1976). This has not been confirmed by other authors (*Harvard, Saldanha, Bird* and *Gardner* 1970). A decreased cortisol clearance rate, resulting from hypothyroidism (*Brien* 1976; *Levin* and *Daughaday* 1955) and an accelerated peripheral metabolism of cortisol, produced by excess thyroid hormone (*Gallagher* et al. 1972) was also involved. Some indirect data from measurements of cortisol secretion episodes and its response to hypoglycemia suggest that ACTH secretion could be the critical factor in these alterations (*Lessof* et al. 1969; *Bigos* et al. 1978; *Gallagher* et al. 1972; *Harvard* et al. 1970). However, the effect of thyroid hormones on pituitary ACTH has not been well established.

Thus, our investigation centered around the regulation of adrenocorticotropin (ACTH) secretion in hyper- and hypothyroid rats through the analysis of circulating corticosterone and ACTH. We also focused on the determination of pituitary ACTH content and the capacity of the pituitary to release ACTH during in vitro incubation. Our data suggest that ACTH secretion is potentiated by thyroid hormones.

Materials and Methods

Animals

Twenty-eight adult male Sprague-Dawley rats (mean weight 262 g) were divided into three groups. Ten animals were injected intraperitoneally (ip) for two weeks with 10 mg L-T_4 every 12 hours; ten were made hypothyroid by the administration of 0.5% 6-propylthiouracil (PTU) in regular iodine containing water ad libitum; eight control rats were injected ip with distilled water. All the animals were fed regular food and water. They were sacrificed by decapitation, between 9 and 10 AM, blood was collected in chilled polystyrene tubes containing 1 mg EDTA per ml of blood and centrifuged at 2500 g for 30 min, between 5 and 15 min after collection.

Pituitary incubation

Pituitaries were removed, placed on tissue culture dishes (60 × 15 mm) containing 3 ml Dulbecco's Modified Eagle's Medium (DMEM) and incubated for 30 min at 37 °C with 95% air, 5% CO_2 in a water jacketed incubator. Following this preincubation, media were discarded, new DMEM was added, and a 3 h incubation period proceeded. Media were collected and pituitaries extracted in Duvall all glass homogenizers, using 1 ml chilled 0.05 M phosphate buffer, pH 7.5 per pituitary. The homogenates were boiled for 5 min and centrifuged at 2500 g for 30 min. Supernatants were kept frozen at –20 °C until assay.

Radioimmunoassays

ACTH was measured in non-extracted incubation media, pituitary and plasma. Each assay tube received 100 μl of sample and 900 μl of assay buffer (0.05 M PO_4, pH 7.6, 0.25% crystalline bovine serum albumin (cBSA), 0.1% mercaptoethanol). The antiserum donated by Dr. West and kindly supplied by the Rat Pituitary Hormone Distribution Program of the NIAMDD at NIH (Bethesda, MD, USA), was used at a final concentration of 1 : 200.000. Synthetic human ACTH (1–39) (NIH-Bethesda) was used for iodination and standards. Standards were prepared in ACTH free rat plasma, obtained by treatment with dexamethasone. Second antibody procedure

Horm. metabol. Res. 21 (1989) 550–552

Received: 8 March 1988 **Accepted: 24 Febr. 1989 after revision**

Table 1 Plasma levels of T_4, T_3 and TSH.

	Control	Hypothyroid (PTU)	Hyperthyroid (T_4)
	(8)	(10)	(10)
T_4 (μg/100 ml)	3.9 ± 0.4	< 2[a]	17.3 ± 2.2 [a]
T_3 (ng/100 dl)	118.7 ± 25.5	< 10[a]	157 ± 46 [b]
TSH (μg/ml)	.39 ± 0.05	1.3 ± 0.2[b]	0.10 ± 0.02

Values are the mean ± SD; the number of animals studied is in parenthesis. Significance vs control values: [a]$P < 0.01$; [b]$P < 0.01$.

was used for separation. The sensitivity of the assay was 29 pg/ml (equivalent to 2.9 pg/tube). The coefficients for intra- and interassay variation were 8% and 17% respectively. All the steps of the assay were done at 4 °C.

TSH was measured by double antibody radioimmunoassay using a National Pituitary Hormone Distribution Program rat hormone kit (NIAMDD, Bethesda, MD, USA), and was expressed in terms of a rat TSH standard (NIAMD-Rat-TSH-RP-1). The rTSH S_3 antiserum was used at a final dilution of 1/75.000. The sensitivity of the assay was 40 ng/ml (2 ng/tube). The coefficients for intra- and interassay variation were 5% and 11% respectively. Corticosterone was measured by competitive protein binding assay, using ^3H labelled steroid (*Murphy* 1967). Thyroxine (T_4) and Triiodothyronine (T_3) were determined by RIA using commercial kits.

Statistical significance of the differences between mean values was calculated by the Student's t-test.

Results

Plasma T_4, T_3 and TSH

Thyroid status was verified by measuring plasma T_4, T_3 and TSH. As indicated in Table I, thyroid hormones were significantly elevated in the T_4 and decreased in the PTU treated rats. To the contrary, TSH was decreased by T_4 treatment and increased by PTU administration. These data confirm the efficiency of the two different treatments on thyroid function.

Plasma ACTH and corticosterone

The effect of thyroid hormones on adrenocortical function was verified by measuring the plasma levels of corticosterone and immunoreactive ACTH. Fig. 1 shows that corticosterone and ACTH were significantly higher in hyperthyroid than in the control rats; ACTH (pg/ml) mean ± SE: control 154 ± 27, T_4 261 ± 49 ($P < 5.6$ ($P < 0.001$). The hypothyroid animals showed a significant decrease in plasma corticosterone, with ACTH also diminished, but not significantly; corticosterone (μg/100 ml) mean ± SE: control 20 ± 3.4, PTU 12 ± 2,1 ($P < 0.05$).

Pituitary ACTH and TSH content and secretion

To figure out whether variations in plasma ACTH merely reflect changes in its secretion or could also be attributed to modifications in its synthesis, pituitary ACTH content and its in vitro secretion capacity were determined. As is shown in Fig. 2, immunoreactive ACTH content was significantly decreased in hypothyroid rats. Hyperthyroid animals

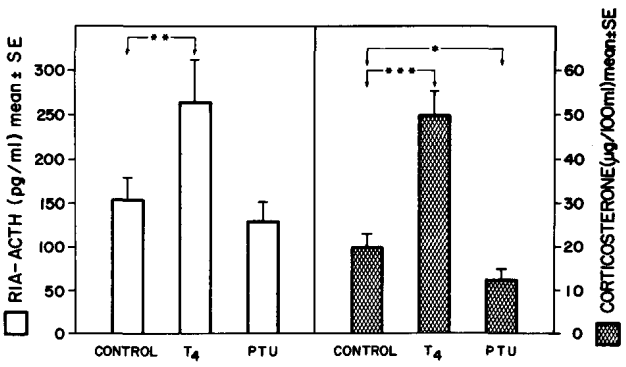

Fig. 1 Plasma ACTH (left panel) and corticosterone (right panel) in euthyroid, hyper- and hypothyroid rats. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

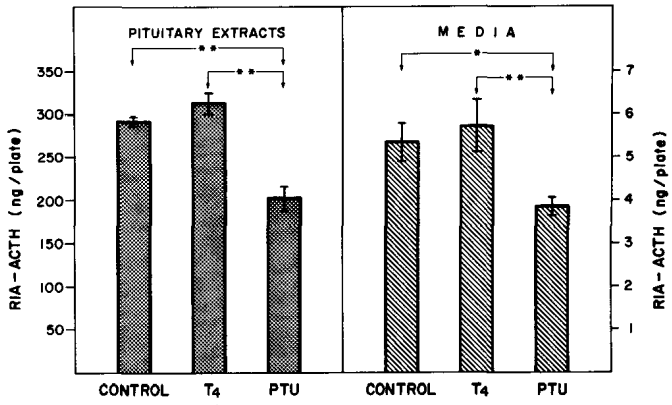

Fig. 2 ACTH content in pituitary extracts and media after 3 h incubation. Values represent mean ± SE of 4 plates, containing 2 pituitaries each. *$P < 0.01$; **$P < 0.005$.

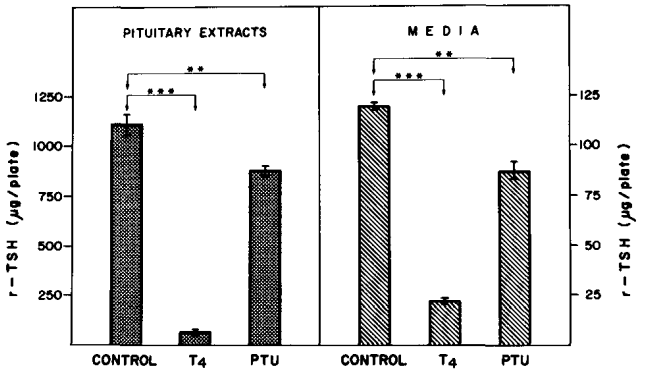

Fig. 3 TSH content in pituitary extracts and media after 3 h incubation. Values represent mean ± SE of 4 plates containing 2 pituitaries each. **$P < 0.01$, ***$P < 0.001$.

had a tendency to increase their pituitary ACTH content, but this was not statistically significant; ACTH (ng/plate) mean ± SE: control 292.4 ± 7.2, T_4 317.6 ± 12, PTU 205.6 ± 10. After 3 h of incubation, ACTH secretion to the medium paralleled pituitary content, decreasing from control

values of 5.4 ± 0.4 to 3.9 ± 0.2 (ng/plate) mean ± SE, $P < 0.05$ in the PTU treated group. TSH content in pituitaries and media after 3 h of incubation is depicted in Fig. 3. T_4 treated rats had a decreased TSH content and secretion, as compared to the controls. TSH content in pituitaries and media was significantly lower in the PTU treated animals than in the control, but higher than in the hyperthyroid group. Pituitary TSH content (μg/plate) mean ± SE: control 1134 ± 63, T_4 62 ± 8, PTU 878 ± 21. Media TSH (μg/plate) mean ± SE: control 119 ± 9, T_4 22 ± 2, PTU 81 ± 7.

Discussion

The data presented in this paper strongly supports the idea that thyroid hormones have a positive regulatory effect on ACTH secretion. The opposite is true for their known action on TSH regulation in the rat. ACTH measurements demonstrating coincidental changes in plasma ACTH and pituitary content and secretion to the medium, along with parallel serum corticosterone modifications, provide evidence that this positive effect is mediated by ACTH.

Previous studies in thyroid disfunction, measuring the plasma corticosteroid response to metyrapone, lysine-vasopressin, hypoglycaemia or exogenous ACTH, suggested that corticoadrenal function is reduced in primary hypothyroidism (*Lessof* et al. 1969; *Bigos* et al. 1978; *Harvard* et al. 1970). However, it is not clear which factors are implicated in this effect, since abnormal thyroid function is associated with variations in corticosteroid metabolism (*Harvard* et al. 1970; *Levin* and *Daughaday* 1955; *Murphy* 1967; *Bassi, Pupi, Gianotti, Fiorelli, Forte, Pinchera* and *Serio* 1980; *Anderson* 1980). Consequently modifications in the renal clearance of steroids could account for these changes. Furthermore, there are data which indicate that corticosteroid binding globulin capacity could be influenced by thyroid hormones, at least under certain conditions and in certain animals, such as fetal sheep and newborn rats (*D'Agostino* and *Henning* 1981; *Ballard, Klein* and *Fisher* 1983).

In the present study, we have clearly established that thyroid hormones increase ACTH secretion and consequently corticosterone production. These data indicate that apart from the influence on corticosteroid metabolism and modifications in corticosteroid binding globulin capacity, thyroid hormones regulate ACTH secretion.

A similar thyroid hormone stimulatory action has been described for other pituitary hormones such as GH and gonadotropins (*Hervás, Morreale* and *Escobar* 1975; *Aranda, Hervás, Morreale* and *Escobar* 1976). This is the opposite of the inhibitory effect which is known to occur on TSH (*Cacicedo, Pohl* and *Reichlin* 1981).

The present data do not allow us to establish whether thyroid hormone action occurs primarily at the pituitary or at the hypothalamic level or at both. The occurrence of more cortisol secretion episodes in hyperthyroid than in normal humans (*Gallagher* et al. 1972) indicates the hypothalamic implication of this effect. Thus far, no data has been presented on corticotropin releasing factor secretion in relation to thyroid hormones.

These data give support to the important role of thyroid hormones in ACTH regulation and, therefore, in the adaptation to stress.

Acknowledgements

We would like to acknowledge the help of Ms. Anna Steele in preparing this manuscript.

This research was supported by the Fondo de Investigaciones Grant 86/819.

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