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INFLUENCE OF THYROID HORMONE ON CORTISOL BIOSYNTHESIS. A GASCHROMATOGRAPHIC ANALYSIS

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SUMMARY

The influence of thyroid hormone on the functional activity of adrenal 11β-hydroxylating enzyme in cortisol biosynthesis was evaluated indirectly by measuring in 43 adult subjects (17 hyperthyroids, 6 hypothyroids and 20 controls) the ratio between the main catabolic product of 11-deoxycortisol, tetrahydrodeoxycortisol (THS), and the sum of the 6 main cortisol catabolic products ("cortisol metabolites"). All these steroids were studied by means of a gas–liquid chromatographic analysis of total neutral fraction on the 24 h urine output. The test was made after corticotrophic stimulation with 1 mg tetracosatrin (Synacthen Depot®) injected intramuscularly. The value of THS/cortisol metabolite ratio was 0.042 $\pm$ 0.006 in hyperthyroids, 0.090 $\pm$ 0.040 in hypothyroids, and 0.040 $\pm$ 0.008 in normal subjects. The difference between hypothyroids and controls was significant ($P < 0.05$). The results are briefly discussed.

INTRODUCTION

Several changes in peripheral cortisol metabolism are known in thyroid disease. Alterations in cortisol secretion rates are said to be caused by parallel changes in metabolic clearance rates; in hyperthyroidism they are accelerated, in hypothyroidism they are, on the contrary, slowed.

Adaptation of the "feed-back" corticotrophic pituitary control mechanism is thought to equalize cortisol secretion to its peripheral biotransformation rate [2,3,5,7–18].

A relation between thyroid function and corticosteroid metabolism is well known; cortisol metabolite excretion is increased in hyperthyroids, diminished in hypothyroids; thyroid hormone affects some specific reactions in androgen peripheral metabolism (balance between 5α-and 5β-reduction of the double bond 4–5–one) [1,18,22] and in cortisol biotransformation (reaction equilibrium of the cortisol/cortisone reversible oxidation-reduction) [6,7,12,18].

As far as we know, nobody up to this time has demonstrated any effect of thyroid hormone on specific reactions in cortisol biosynthesis, except ones under ACTH control (cholesterol → 5-pregnenolone transformation). This work is concerned with cortisol biosynthesis at a level beyond this stage: 11β-hydroxylation.

EXPERIMENTAL

Materials

Forty three adult subjects were studied. Seventeen were hyperthyroid (3 males and 14 females aged 16–63 years), 6 were hypothyroid (all females, aged 43-67 years), 20 were healthy subjects with a normal thyroid function (8 males and 12 females, aged 25–52 years).

Twenty-four hour urine was collected from every patient after corticotrophic stimulation with 1 mg tetracosatrin (Synacthen Depot®) injected intramuscularly; a small urine sample (about 50 ml) was stored at −20°C until assayed.

Methods

The method adopted was described by Ros *et al.*[19–21] and is briefly described here.

One-thousandth of the 24-h urine output, increased in volume to 2 ml, was incubated with β-glucuronidase and sulphatase at pH 5.2 with acetate buffer at 37°C for 48 h. After hydrolysis, 1 μg of oestratetraenol (1,3,5 (10),16-oestratetraene) and 2 μg of cholesterol butyrate were added as internal standards. The pH was adjusted to 12.5 and the steroids extracted twice with 15 ml of diethyl ether. After washing with 0.5 ml of carbonate buffer at pH 10.5, and drying with anhydrous Na_2SO_4, the ether was evaporated to dryness under nitrogen flow at 42°C. To the dry extract were added 5–10 mg of potassium acetate and 100 μg of BSTFA [N,O-bis (trimethysilyltrifluoro-acetamide)]; silylating reaction (23,4) [base-catalysed formation of tri-methylsilyl (TMS) and enol-TMS derivatives of steroids] developed at 62°C for 11 min was interrupted by cooling and drying under nitrogen flow. Then the dry residue was dissolved in 100 μl of hexamethyldisilazane.

Sampling was made by filling small capillary tubes with a 2 μl capacity; these were dried under nitrogen flow and put in a G.L. chromatographic device "FRACTOVAP 2400 T", Carlo Erba, equipped with

Table 1. List of abbreviations for metabolic derivatives of Reichstein's substance S and cortisol

Abbreviation	Trivial name	Systematic name
THS	11-deoxy-tetrahydrocortisol	3α,17,21-trihydroxy-5β-pregnan-20-one
THE	Tetrahydrocortisone	3α,17,21-trihydroxy-5β-pregnane-11,20-dione
THF	Tetrahydrocortisol	3α,11β,17,21-tetrahydroxy-5β-pregnan-20-one
CTE	Cortolone	3α,17,20α,21-tetrahydroxy-5β-pregnan-11-one
β-CTE	β-Cortolone	3α,17,20β,21-tetrahydroxy-5β-pregnan-11-one
CTO	Cortol	5β-pregnane-3α,11β,17,20α,21-pentol
β-CTO	β-Cortol	5β-pregnane-3α,11β,17,20β,21-pentol

an automatic sampler: sampling was made with a flash heater at 270°C.

A capillary glass column (25 m long, with an internal diameter of 0.31 ± 0.01 mm and an internal coat of O.V.-101) was employed. Hydrogen was employed as a carrier gas; running conditions were as follows: flow was 1.6–2.1 ml/min; column temperature was electronically programmed (during the sampling phase it was at 55°C; in following 2 min it fell to 45°C, then in 5 min rose to 170°C, remained constant for 10 min and later increased regularly at a rate of 1.8°C/min to 248°C).

A flame ionization detector was used.

The relationship between the quantity of single steroid compounds and corresponding peak heights in chromatogram was evaluated by processing, throughout the above method, a mixture of pure steroid (2 μg each) resuspended in 2 ml of distilled water. All steroids taken into account in the G.L. chromatographic analysis were represented in the aqueous suspension. This procedure was repeated 10 times and the "theoretic peak height" for 2 μg of each steroid was calculated [21]; this value was dependent on the recovery of the individual steroid; for every one of them there was an almost constant loss due to its polarity and to different ether–water partition coefficients. The loss was very small for the less polar steroids, for instance, androsterone and pregnandiol (1–2%), whereas for the most polar, like cortolone and cortol, it ranged from 30% to 35%. However, even this large loss proved very constant. Loss due to ether–water partition was calculated once by dissolving the mixture of pure steroids directly in ether, and by evaluating the ratio between peak height obtained starting from water and peak height obtained starting from ether [21].

Sampling error was calculated by adding to every sample two internal standards, oestratetraenol and cholesterol butyrate (see above) [21].

Twenty-one different steroid compounds with 19 and 21 Carbon atoms were detected in every sample. Only 7 (Table 1) are taken into account in this work.

The efficiency of the conversion of Reichstein's compound S to cortisol was evaluated indirectly both by measuring the main catabolic product of 11-deoxy-cortisol, THS, and by calculating the ratio between this compound and total urinary excretion of the 6 main catabolic products of corisol, THE, THF, cortolone, β-cortolone, cortol, β-cortol; the sum of the latter substances is designated "cortisol metabolites".

THS was measurable only after corticotrophic stimulation; in fact, without stimulation, urinary excretion of this compound was minimal and not suitable for measurement.

Results, expressed as mean ± SE, were evaluated with Student's *t*-test.

RESULTS

After corticotrophic stimulation, urinary excretion of cortisol metabolites was 20.98 ± 2.68 mg/24 h in

Table 2. Urinary excretion of THS and main cortisol catabolic products, expressed as mg/24 h, in hyperthyroid, hypothyroid, and control subjects after corticotrophic stimulation (Synacthen Depot R, 1 mg)

	Controls (No.16)	Hyperthyroids (No.15)	Hypothyroids (No.6)
THS	0.62 ± 0.13	0.92 ± 0.13*	1.49 ± 0.42*
THE	4.07 ± 0.50	7.34 ± 0.90**	3.78 ± 1.30
THF	6.34 ± 0.75	6.19 ± 0.95	7.49 ± 2.36
CTE	1.75 ± 0.22	3.52 ± 0.45**	1.39 ± 0.45
β-CTE	0.91 ± 0.11	1.98 ± 0.30**	0.64 ± 0.21
CTO	0.63 ± 0.07	0.92 ± 0.19	0.92 ± 0.35
βCTO	0.89 ± 0.15	1.01 ± 0.20	0.92 ± 0.35
Cortsisol metabolites	14.61 ± 1.58	20.98 ± 2.68	15.14 ± 4.91
THS/Cor.met.	0.040 ± 0.008	0.042 ± 0.006	0.090 ± 0.04*

The sum of the THE, THF, CTE, β-CTE, CTO, β-CTO is designed "cortisol metabolites".

*$P < 0.05$ **$P < 0.005$

hyperthyroids, 15.14 ± 4.91 mg/24 h in hypothyroids, and 14.61 ± 1.58 mg/24 h in normal subjects. No statistical difference between various groups was apparent.

THS urinary excretion was 0.92 ± 0.13 mg/24 h in hyperthyroids, 1.49 ± 0.42 mg/24 h in hypothyroids, and 0.62 ± 0.13 mg/24 h in normal subjects. Statistical difference between hypothyroids and controls, and between hyperthyroids and controls was significant ($P < 0.05$).

The value of THS/cortisol metabolite ratio was 0.042 ± 0.006 in hyperthyroids, 0.090 ± 0.040 in hypothyroids, and 0.040 ± 0.008 in normal subjects. The difference between hypothyroids and control subjects was statistically significant ($P < 0.05$) (Table 2).

Therefore, the THS levels both in hypothyroid and hyperthyroid subjects were higher than in controls. However, in hyperthyroidism, but not in hypothyroidism, there was a parallel increase in cortisol metabolites, so that THS/cortisol metabolite ratio was high in the hypothyroidism condition only, showing the existence of a diminution in the function of 11β-hydroxylase.

DISCUSSION

It is well known that after corticotrophic stimulation normal subjects produce an increased quantity of compound S [10] as a consequence of a partial functional insufficiency of the conversion of this substance to cortisol, as revealed by the activation of the ACTH-promoted conversion of cholesterol to 5-pregnenolone.

This phenomenon appeared markedly accentuated in hypothyroid subjects, as shown by measuring urinary THS/cortisol metabolite ratio; therefore the existence of a partial block of the 11β-hydroxylase enzymatic system became evident.

Until now, the influence of thyroid hormone on cortisol biosynthesis has appeared to be exclusively indirect, based on the effects on extra-adrenal (mainly hepatic) cortisol metabolism, and on changes of ACTH plasma levels.

Our data reveal a direct action of thyroid on adrenals; a normal thyroid hormone plasma level appears to be necessary for a normal activity of 11β-hydroxylase.

The role of this enzymatic block is, however, secondary in determining the known changes of cortisol metabolism in hypothyroidism; the phenomenon has a mainly speculative interest.

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