Effects of Thyroid Hormones on Ring A Reduction of Cortisone by Liver

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ABSTRACT

Yates, F. Eugene, John Urquhart and Arthur L. Herbst. Effects of thyroid hormones on ring A reduction of cortisone by liver. Am. J. Physiol. 195(2): 373-380. 1958.—Triiodothyronine increases total hepatic capacity for in vitro reduction of ring A of cortisone by 38% in both male and female rats. Thyro-parathyroidectomy decreases it 56% in males and 48% in females. These alterations in thyroid state influence hepatic reduction of corticosteroids in several ways: a) the total amount of \( \Delta^4 \)-steroid hydrogenases in the liver may be altered, both by changes in liver size and by changes in amount of enzyme per gram of liver; and, b) the activity per gram of liver can also be altered through variations in coenzyme (TPNH) availability. These effects of thyroid hormones provide an enzymatic basis for the alterations in biological half-life of adrenal steroids observed in hyper- and hypothyroidism. A very close correlation \( (r = 0.97) \) between total hepatic capacity to inactivate cortisone and the size of the adrenal glands was found. It is suggested that the rate of ring A reduction of corticosteroids by liver determines the rate of ACTH secretion in unstressed animals.

In several species it has been shown that the rate of disappearance from plasma of administered adrenal cortical hormones is increased in hyperthyroid states and decreased in hypothyroid states (2-5). Since alterations of renal excretion of unmetabolized steroids are not sufficient to account for these changes in the half life of the adrenal cortical hormones (3), it has been concluded that thyroid hormones increase the rate of inactivation of corticosteroids. The present study was undertaken to determine whether thyroid hormones affect the rate of hepatic reduction of ring A of adrenal steroids and, if so, whether a specific hormonal induction of the \( \Delta^4 \)-steroid hydrogenases is involved.

METHODS

Adult male and female Sprague-Dawley rats weighing approximately 250 gm and main-

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2 See reference (1).
nate assay was designed to measure only changes in the amount of the $\Delta^4$-steroid hydrogenases in the liver tissue. Comparison of the results from the two different assays of the same tissue was used to differentiate the effects of thyroid hormones on the amount of the enzymes themselves, from other effects such as changes in cofactor availability, which might also alter the reaction rate in hepatic cells.

For all assays rats were killed by a blow on the head and the livers were quickly excised, rinsed free of gross blood in iced saline, blotted and weighed. A 5-gm aliquot was taken immediately to be assayed in duplicate as homogenate according to the method we have previously described in detail (6). From another aliquot thin slices were cut on a Stadie-Riggs hand microtome. Slice weights ranged from 100 to 250 mg. One gram of slices ($\pm 0.015$ gm) were added to 10 ml of warmed oxygenated Krebs' phosphosaline buffer, pH 7.40, containing 16.4 mg glucose, 3.33 $\mu$moles steroid, and 0.12 ml absolute ethanol. This reaction mixture was incubated at 37.5°C under 100% oxygen with intermittent shaking for 15 minutes. For each flask incubated at 37.5°C a duplicate was held at 0°C. The contents of both vessels were subsequently treated similarly. At the end of the incubation, 245 volumes of acetone were added, and the vessel was allowed to stand for 1-3 hours. The slices were then pulverized with a glass rod and the contents of the vessel were transferred to a centrifuge tube and spun at 3000 $\times$ g for 20 minutes. The resultant supernatant was decanted with rinses and brought to a known volume with acetone. An aliquot was transferred to a separatory funnel containing one-fourth volume distilled water, and extracted with $1\frac{1}{2}$ volumes of redistilled dichloromethane. The remaining aqueous phase was extracted twice more with dichloromethane and discarded. The combined dichloromethane-acetone extracts were washed once with 0.1 $\rm{N}$ HCl. The remaining acid phase was backwashed with 2 volumes of dichloromethane, and the backwash was combined with the washed extract. The mixture was evaporated to dryness under nitrogen jets in a water bath. The residue was redissolved in 10 ml of absolute methanol for analysis at 240 $\mu$m in a Beckman model DU spectrophotometer with matched 3 ml silica cuvettes. Absolute methanol was used as reference.

The quantity of steroid reduced at the $\Delta^4$ group of ring A was calculated from the difference between the optical densities of the extracts of 0°C and 38°C incubation mixtures. The reaction velocities for both the homogenate and slice assays are given as $\mu$moles $E$ reduced/gram liver (wet weight)/15 minutes. The total hepatic capacity for the in vitro reduction of ring A is derived from the product of the metabolism measured in the slice assay and the liver weight expressed as percentage of total body weight. Data presented as means are accompanied by the standard error of the means.

RESULTS

Background Readings and Recovery of Unmetabolized Substrate. The background readings of extracts from liver preparations without added steroid did not show an absorption maximum in the range 220-260 $\mu$m. Therefore no endogenous steroids with an intact $\Delta^4$-3-keto group were detected. The background reading from homogenate preparations was very low, as previously reported, and recovery of known amounts of added steroid from mixtures in which metabolism had been prevented was 95% complete.

In the slice preparations the absolute optical density of the extracted background material was independent of both temperature of incubation and previous thyroid treatment of the animal, and recovery of known amounts of steroid from the reaction mixtures held at 0°C for 15 minutes was 86.0 $\pm$ 0.5%. Since the amount of metabolism was calculated from the difference in optical density at 240 $\mu$m between the 0°C and the 37.5°C mixtures, systematic errors introduced by tissue background and incomplete recovery of unmetabolized steroid were minimized by cancellation.

Characteristics of the Assays. The reaction velocity observed in the homogenate assay was constant during the 6-minute assay period. It was linearly related to the amount of enzyme present over the ranges studied and was independent of substrate and cofactor (reduced triphosphopyridine nucleotide) concentrations (6). Under these conditions quantitative com-
FIG. 1. Homogenate data are a measure of the concentration of A4-steroid hydrogenase activity in the liver. The slice data are a measure of the capacity of a given weight of liver to carry out the reaction catalyzed by the hydrogenase, as it is determined both by the concentration of the enzyme and by the availability of coenzyme within the cells, and possibly by other factors as well.

Comparisons of tissue enzyme concentrations were possible. In the slice assay approximately one-third of the total amount of ring A reduction observed after 60 minutes of incubation occurred within the first 15 minutes. The amount of metabolism per gram of liver was not influenced by variations in mean weight of individual slices in the reaction mixture.

Sex Difference in the Rate of Ring A Reduction of Cortisone by Normal Livers. Both assays demonstrated a larger capacity for the A4 reduction of cortisone in the female (fig. 1). The ratio of female to male activity observed in the homogenate assay was 2.5 and the ratio observed in the slice assay was 2.0. Livers from control females assayed as homogenate reduced 3.2 ± 0.14 μM E/gm/15 min. and when assayed as slices reduced 1.8 ± 0.10 μM E/gm/15 min. Livers from control male animals reduced 1.3 ± 0.10 μM E/gm/15 min. as homogenates, and 0.94 ± 0.048 μM E/gm/15 min. as slices. Livers from females represented 0.04% of the respective body weights, so the sex difference in total hepatic activity for A4 reduction of cortisone was also large. This sex difference in steroid metabolism has been previously described for other substrates as well (6), and is being reported in detail elsewhere.

Hyperthyroid Groups. Livers from male rats treated with 20 μg/day of triiodothyronine for 12 days showed a 34% increase in the rate of reduction of cortisone per gram of liver in the slice assay (P < 0.01), without a detectable change in tissue enzyme concentration as measured by the homogenate assay (fig. 1). A small increase in liver size relative to body weight (table 1) contributed to the 38% increase in total hepatic activity in this group (fig. 2). In a group of six males receiving only 10 μg/day of triiodothyronine for 12 days the activities of the slices, homogenates and total livers all remained at control levels.

In livers from females receiving the 20-μg dose for 12 days there was no significant change in the activity per gram of liver in either the slice or homogenate assay (fig. 1). However,
### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt. at Time of Assay</th>
<th>Adrenal</th>
<th>Thyroid</th>
<th>Liver</th>
<th>Liver as % Body Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>gm</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>208 ± 5</td>
<td>38.9 ± 1.9</td>
<td>16.3 ± 0.78</td>
<td>11.2 ± 0.25</td>
<td>3.8 ± 0.04</td>
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<tr>
<td>N = 18</td>
<td></td>
<td>N = 18</td>
<td>N = 17</td>
<td>N = 18</td>
<td></td>
</tr>
<tr>
<td>10 µg Triiodothyronine (TIT)</td>
<td>315 ± 13</td>
<td>45.6 ± 1.2</td>
<td>13 ± 0.307</td>
<td>12.3 ± 2.1</td>
<td>3.0 ± 0.12</td>
</tr>
<tr>
<td>N = 8</td>
<td></td>
<td>N = 8</td>
<td>N = 6</td>
<td>N = 8</td>
<td></td>
</tr>
<tr>
<td>20 µg TIT</td>
<td>253 ± 12</td>
<td>51.5 ± 1.9</td>
<td>14.3 ± 3.1</td>
<td>9.8 ± 0.69</td>
<td>3.9 ± 0.09</td>
</tr>
<tr>
<td>N = 14</td>
<td></td>
<td>N = 14</td>
<td>N = 5</td>
<td>N = 14</td>
<td></td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>302 ± 13</td>
<td>33.1 ± 1.33</td>
<td>9.2 ± 0.31</td>
<td>3.12 ± 0.2</td>
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<td>N = 9</td>
<td>N = 8</td>
<td>N = 8</td>
<td></td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>259 ± 10</td>
<td>61.5 ± 3.9</td>
<td>16.6 ± 1.96</td>
<td>8.75 ± 0.35</td>
<td>3.4 ± 0.07</td>
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<td></td>
<td>N = 10</td>
<td>N = 4</td>
<td>N = 9</td>
<td></td>
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<tr>
<td>20 µg TIT</td>
<td>240 ± 10</td>
<td>79.5 ± 2.4</td>
<td>12.2 ± 0.9</td>
<td>11 ± 0.35</td>
<td>4.6 ± 0.14</td>
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<td></td>
<td>N = 10</td>
<td>N = 7</td>
<td>N = 10</td>
<td></td>
</tr>
<tr>
<td>20 µg Thyroxine</td>
<td>262 ± 9</td>
<td>70.8 ± 2.04</td>
<td>10.5 ± 0.6</td>
<td>9.3 ± 0.36</td>
<td>3.5 ± 0.08</td>
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<tr>
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<td></td>
<td>N = 6</td>
<td>N = 6</td>
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<td></td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>270 ± 4</td>
<td>44.9 ± 6.75</td>
<td>8.5 ± 0.24</td>
<td>3.15 ± 0.58</td>
<td></td>
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<tr>
<td>N = 11</td>
<td></td>
<td>N = 11</td>
<td>N = 11</td>
<td>N = 11</td>
<td></td>
</tr>
<tr>
<td>Thyroidectomized + 20 µg TIT</td>
<td>275 ± 18</td>
<td>59.6 ± 3.4</td>
<td>9.5 ± 0.5</td>
<td>3.5 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>N = 4</td>
<td></td>
<td>N = 4</td>
<td>N = 4</td>
<td>N = 4</td>
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</tr>
</tbody>
</table>

Organ weights and body weights. Not all the animals from which organ weight data were obtained were used in the assay experiments. The organ weight changes referred to in the text are concerned with data obtained from animals actually studied in the assay experiments, and are included in those shown above.

In these animals the livers were considerably enlarged (table 1) so the total hepatic activity was actually 38% greater than that of the control animals of the same age and weight (fig. 2).

Since triiodothyronine failed to affect the amount of metabolism by slices in the females, though it had done so in the males, the experiment was repeated in a group of six females which had received 20 µg/day of thyroxine for 12 days. Again no change in hepatic activity per gram of liver was observed in either assay.

**Hypothyroid Groups.** There was no evidence of hypoparathyroidism in any of these animals. Livers from male rats studied two months after thyro-parathyroidectomy showed a 33% decrease in the rate of reduction of ring A of cortisone in the slice assay \(P < 0.01\). However, once again no change in the amount of \(\Delta^4\) hydrogenases in the tissue had occurred (fig. 1—homogenates). The livers from these animals were smaller than those of control animals and the loss of total hepatic activity in the hypothyroid males was 56% of the control values \(P < 0.01\) (fig. 2).

In contrast, livers from female rats studied 3 weeks to 3 months after thyro-parathyroidectomy showed a 31% decrease in the concentration of the enzyme itself \(P < 0.01\) (fig. 1). Liver slices from this group showed a 43% loss of activity per gram, and total hepatic activity was decreased significantly by 48% \(P < 0.01\) (fig. 2). The duration of the hypothyroidism was unimportant: the results were the same for the 3 week group (5 animals) as for the 3-month group (4 animals).

**Treatment of Hypothyroid Rats With Triiodothyronine.** Since the response of animals to exogenous hormones is often enhanced after removal of the endogenous source, we repeated the 20 µg/day triiodothyronine treatment with a group of four female rats which had been thyroidectomized for three months. Once again the treatment failed to alter the concentration of the enzyme in the liver, but did result in a significant increase in the activity of slices (fig. 1). The velocity of the reaction in this assay increased from a low of 57% of control values in the untreated hypothyroid females to 85% of control levels in the triiodothyronine-treated thyroidectomized group, even though the enzyme concentration remained low and unchanged.

The possibility that thyroid hormones might
caused a severe loss of the enzyme in both sexes. The hyperthyroid females had more of the enzyme in their livers, but there was no significant change in the hyperthyroid males, even though the total hepatic activity measured by the slice assay was significantly elevated (fig. 2).

Changes in Thyroid State and the Sex Difference in Metabolism. As is shown in figures 1 and 2 the sex difference in cortisone metabolism was present in all comparably treated male and female groups, and was not abolished by changes in the thyroid states of the animals. However thyroidectomy caused a decrease in the concentration of the enzyme in livers of females, while it had no effect in males, so the sex difference was thereby diminished in hypothyroid animals.

Correlation Between Changes in Total Hepatic Activity for \( \Delta^1 \) Reduction of Cortisone and Changes in Adrenal Weight. In both sexes the hyperthyroid groups had larger adrenals and the hypothyroid groups had smaller adrenals than did control animals of similar age or weight (table 1). In addition, the well-known sex difference in adrenal weight in the rat was again observed. There was an excellent correlation \((r = 0.97)\) between the total capacity of the liver for the \( \text{in vitro} \) inactivation of cortisone by reduction of ring A, and the size of the adrenal gland in nine experimental groups representing data obtained from 77 animals (fig. 3). The correlation of hepatic activity with adrenal size for the individuals comprising the control male and female groups was also good \((r = 0.85)\), and the slopes of the regressions of adrenal weight on total hepatic activity did not differ significantly in the two sexes.

**DISCUSSION**

The shortened half-life of adrenal cortical hormones in the plasma of hyperthyroid patients and animals might be accounted for in several ways. An increase in liver blood flow or permeability to steroid molecules could be responsible. Changes in the amount of one or several enzymes or coenzymes concerned with inactivation of adrenal cortical hormones within liver and peripheral tissues might also be a sufficient explanation.

Since the liver accounts for a large part of the total adrenal steroid metabolism in most
Table 2. Total hepatic enzyme content relative to control values

<table>
<thead>
<tr>
<th>Group</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>20 µg Triiodothyronine</td>
<td>106</td>
<td>134</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>68</td>
<td>60</td>
</tr>
</tbody>
</table>

(P < 0.01)

Total hepatic enzyme content was calculated from the product of the activity per gram of liver homogenate and the total liver weight (expressed as percentage body weight). The control values for each sex were arbitrarily set at 100 U. The female control value was actually 2.5 times greater than the absolute value of the male control group. The P values refer to the significance of the difference between the figure for the experimental group and that for the control group for the same sex.

Invertebrate species studied, it seemed reasonable to assume that the thyroid hormones exert their effects upon steroid metabolism through some change in hepatic function. The reduction of ring A of the Δ4-3-keto steroids was chosen for study because it is known to precede reduction of the 3-ketone and conjugation (7,8) and it is a major reaction in the inactivation of adrenal cortical hormones.

In these experiments the rate of reduction of corticosteroid substrates in hepatic cells, at a given initial substrate concentration, appears to be some function of the amount of enzyme in the tissue. All differences in tissue enzyme concentration, observed among the various experimental groups by homogenate assay, were associated with similar differences in the rate of steroid metabolism by slices. However, that the amount of enzyme present is not the only factor determining the reaction rate within the cells at a given substrate concentration is shown by the three instances in which large changes in slice activity occurred at constant tissue enzyme concentration. Since the in vitro addition of reduced triphosphopyridine nucleotide (TPNH) to liver slices causes a significant and immediate increase in reaction rate (9), it appears that changes in coenzyme availability could account for this dissociation of slice activity and tissue enzyme concentration in these experiments.

Two stereospecific enzymes exist for the ring A reduction of Δ4-3-keto steroids (10) and so another interpretation of this dissociation might be that the slice assay measured activity of one, and the homogenate assay measured activity of the other. The ring A reduction of desoxycorticosterone by rat liver slices has been reported to lead to the 5α (A/B trans) end-products (11). Rat liver crude homogenates metabolize Δ4-androstene-3,17-dione to the 5α end-products (12) and apparently also convert adrenal cortical steroids predominantly to the 5α compounds (13). However, rat liver in vivo produces significant amounts of the 5β pregnane isomers also (14). From the available evidence it is not yet certain whether the activity of the Δ4,5β steroid hydrogenases is measured by slices and crude homogenates, whereas it is certain that both assays measure the activity of the 5α enzymes. At present no support exists for the view that slices and crude homogenates of rat liver reduce ring A of Δ4,3-keto steroids by completely different pathways. We have therefore assumed that changes in slice activity at constant homogenate activity indicate an alteration in cofactor availability within hepatic cells.

The administration of triiodothyronine or thyroxine to normal animals or to thyroidectomized animals under the various conditions described failed to cause any change in the concentration of Δ4-steroid hydrogenases in the liver. Nevertheless in both sexes hyperthyroidism resulted in an increase in the total hepatic capacity to reduce ring A of cortisone (fig. 2). This response of the hyperthyroid animals was brought about entirely by effects of the thyroid hormones on liver size and coenzyme availability.

The diminution of total hepatic activity in the thyroidectomized males was also brought about entirely by changes in liver size and cofactor availability. However, in contrast to all the other results, the observations made on the thyroidectomized females revealed a significant change in the concentration of the enzyme. In these animals the amount of enzyme per gram of liver was only 70% of control levels (fig. 1).

It appears that alterations in thyroid state can influence hepatic reduction of ring A of adrenal steroids in several ways: a) the total amount of enzyme in the liver can be altered (table 2), both by changes in liver size and changes in the amount of enzyme per gram of
liver; and b) the activity per gram of liver can be altered without a change in the amount of enzyme present—presumably through variations in cofactor availability (fig. 1). In spite of these multiple effects of thyroid hormones on the liver, and the differences in responses of the two sexes, the total hepatic capacity for inactivation of cortisone by ring A reduction was increased in hyperthyroid rats and decreased in hypothyroid rats of both sexes (fig. 2). Such consistent effects of thyroid hormones on the total hepatic activity support the view that there is an enzymatic basis for the in vivo observations that thyroid hormones accelerate the removal of biologically active adrenal cortical hormones from the blood. In addition to the effects noted in these experiments, thyroid hormones can also influence the ratio of 5α to 5β end-products following ring A reduction of testosterone in vivo (15).

The very close correlation between the size of the adrenals and the total in vitro capacity of the liver to inactivate cortisone by reduction of ring A (fig. 3) may be interpreted several ways. Although cortisone is not the predominant steroid secreted by the rat adrenal, preliminary experiments indicate that the relationship holds for corticosterone also. The sex difference in adrenal weights, as well as the changes induced by alterations in thyroid state involve the adrenal cortex and not the medulla (16).

It is possible that the correlation described may represent an independent but quantitatively similar response of the adrenals and the liver to some unrecognized common stimulus. However, if a cause and effect relationship between adrenal size (or secretory rate) and the capacity of the liver to inactivate adrenal steroids exists, it is then necessary to determine which is the independent variable. It could be assumed, for example, that the capacity of the liver for corticosteroid inactivation largely determines the rate of adrenocorticotropic hormone secretion required to maintain normal plasma concentration of corticosteroids. Such
an interpretation of these data offers an explanation for the observations that in hyperthyroidism the half-life of adrenal steroids is shortened; the rate of adrenal cortical secretion is increased, but only if the pituitary is present (5); the adrenals are frequently enlarged and the concentration of the steroids in the blood does not change (4).

The converse assumption, that the hepatic capacity for steroid ring A reduction is dependent upon the rate of adrenal secretion, is equally admissible, but we have so far been unable to demonstrate substrate induction of these steroid hydrogenases in animals with intact adrenals. However, adrenal cortical hormones could increase the availability of the coenzymes required for corticosteroid inactivation, as these experiments indicate that the thyroid hormones do. If so, substrate induction of the enzymes involved would not be a necessary condition for this interpretation of the striking correlation shown in figure 3. Our data at present do not exclude any of these three possibilities.

REFERENCES