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The Thyroid Gland Is a Major Source of Circulating T₃ in the Rat

Jean-Pierre Chanoine, Lewis E. Braverman, Alan P. Farwell, Marjorie Safran, Sharon Alex, Susan Dubord, and Jack L. Leonard

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Abstract

In rats, the respective contribution of the thyroid and peripheral tissues to the pool of T₃ remains unclear. Most, if not all, of the circulating T₃ produced by extrathyroidal sources is generated by 5'-deiodination of T₄, catalyzed by the selenoenzyme, type I iodothyronine 5'-deiodinase (5'D-I). 5'D-I in the liver and kidney is almost completely lost in selenium deficiency, resulting in a marked decrease in T₄ deiodination and an increase in circulating T₃ levels. Surprisingly, circulating T₃ levels are only marginally decreased by selenium deficiency. In this study, we used selenium deficiency and thyroidectomy to determine the relative contribution of thyroidal and extrathyroidal sources to the total body pool of T₃. Despite maintaining normal serum T₃ concentrations in thyroidectomized rats by T₄ replacement, serum T₃ concentrations remained 55% lower than those seen in intact rats. In intact rats, restricting selenium intake had no effect on circulating T₃ concentrations. Decreasing 5'D-I activity in the liver and kidney by > 90% by restricting selenium intake resulted in a further 20% decrease in serum T₃ concentrations in the thyroidectomized, T₄ replaced rats, suggesting that peripheral T₄ to T₃ conversion in these tissues generates approximately 20% of the circulating T₃ concentrations. While dietary selenium restriction markedly decreased intrahepatic selenium content (> 95%), intrathyroidal selenium content decreased by only 27%. Further, thyroid 5'D-I activity actually increased 25% in the selenium deficient rats, suggesting the continued synthesis of this selenoenzyme over selenoproteins in other tissues in selenium deficiency. These data demonstrate that the thyroid is the major source of T₃ in the rat and suggest that intrathyroidal T₄ to T₃ conversion may account for most of the T₃ released by the thyroid. (J. Clin. Invest. 1993. 91:2709-2713.) Key words: deiodination • selenium • thyroid • hormone metabolism • T₄ to T₃ conversion

Introduction

In mammals, the relative contribution of thyroidal and extrathyroidal sources to the total body pool of the metabolically active iodothyronine, 3,5,3'-triiodothyronine (T₃), is unclear. T₃ can be derived from conversion of the prohormone thyroxine (T₄) by outer ring (5'-) deiodination in the peripheral tissues, by T₄ to T₃ conversion within the thyroid gland, and by direct secretion of de novo synthesized thyroidal T₃. Estimates of the contribution of extrathyroidal T₄ to T₃ conversion to the total T₃ pool vary from 20% to 100% in the rat (1-3) and the thyroid accounts for the remainder of the T₃ produced daily. Lauberg used in situ thyroid perfusion to directly examine the contribution of the thyroid to the T₃ pool and reported that intrathyroidal T₄ to T₃ conversion accounted for a considerable portion of the T₃ secreted from the dog thyroid (4, 5). Thus, both extrathyroidal and intrathyroidal T₄ to T₃ conversion appear to participate in the daily production of T₃. T₄ to T₃ conversion is catalyzed by the enzyme, iodothyronine 5'-deiodinase. Two isozymes of iodothyronine 5'-deiodinase have been identified. The most abundant form, type I iodothyronine 5'-deiodinase (5'D-I), is found in liver, kidney, and thyroid (6) and contains the rare amino acid selenocysteine (7-9). Tissue content of 5'D-I in the liver and kidney is proportional to selenium intake (10). The other isozyme, type II iodothyronine 5'deiodinase (5'D-II), is abundant in the brain, pituitary, and brown adipose tissue and does not contain selenium (11, 12). T₃ generated by 5'D-I is released into the general circulation, while the majority of the T₃ produced by 5'D-II remains with the cell. The ability to manipulate 5'D-I levels by altering the dietary intake of selenium provides the means to examine the contribution of 5'D-I to total T₃ production. Selenium deficiency leads to an almost complete loss of 5'D-I in the liver and kidney and a 40-50% increase in the serum T₄ concentration (10, 12-14). This increment in circulating T₄ is completely accounted for by the prolonged metabolic half-life of the iodothyronine due to the loss of 5'D-I (14). Paradoxically, serum T₃ concentrations are not reciprocally affected and decrease by no more than 20%, if at all (12-14). While serum T₃ concentrations are marginally depressed by selenium deficiency, circulating T₃ sulfate concentrations increase nearly twofold (12, 14). Serum TSH levels remain near normal despite the elevated circulating T₄ in the selenium-deficient animal (12-14). Thus, despite the marked decrease in hepatic and renal T₄ to T₃ conversion in the absence of selenium, other sources of T₃ appear to be made available in animals lacking 5'D-I.

There are several possibilities to account for the discordance between the near complete loss of 5'D-I and the marginal fall in circulating T₃ observed in selenium deficiency. They include (a) diminished T₃ clearance, (b) increased thyroid T₃ secretion, and/or (c) enhanced recovery of T₃ from sulfo-conjugates released into the gut in the enterohepatic cycle. Previous work has shown that T₃ clearance is only marginally decreased by selenium deficiency and that the 20-25% increase in the metabolic half-life of this iodothyronine is insuffi-
cient to maintain the steady-state levels of T₃ observed in serum (14). The contribution of the thyroid to the T₃ pool in the selenium-deficient rat remains to be determined. Likewise, the contribution of enterohepatic recycling of T₃ or its conjugates to the circulating T₃ pool is unclear.

In this study, we determined the source(s) of circulating T₃ in selenium-deficient rats. The data show that the thyroid gland serves as a major source of circulating T₃ in the rat and suggest that intrathyroidal T₄ to T₃ conversion accounts for much of the T₃ secreted by the thyroid.

Methods

Animals and reagents. Weanling male Sprague-Dawley rats (40–50 g) supplied by Charles River Laboratories (Wilmington, MA) were used in all experiments. The study was approved by the Animal Research Committee and complies with the institutional assurance certificate of the University of Massachusetts Medical Center. Rats were fed a torula yeast based semisynthetic diet (Teklad Premier Laboratory Diets, Madison, WI) for 5 wk. The selenium-deficient diet (TD 86298) contains less than 16 μg/kg selenium and the selenium-replete diet (TD 91259) is the same base diet supplemented with 200 μg/kg selenium as Na₂SeO₃. Rats were housed in stainless steel cages, and distilled water was available ad lib. Body weight (BW) was monitored biweekly.

Analytical procedures and hormone assays. In all experiments, animals were killed by decapitation and exsanguinated, except where noted. Liver was homogenized in 4 vol (wt/vol) of 20 mM potassium phosphate buffer (pH 7.4), 150 mM NaCl, and in 4 vol (wt/vol) of 250 mM sucrose, 20 mM Heps buffer (pH 7.0), 1 mM EDTA, and 1 mM DTT and stored at −20°C for determination of glutathione peroxidase activity (GPx) and 5'D-I activity, respectively. Thyroid glands were weighed and homogenized in 800 μl of 250 mM sucrose, 20 mM Heps buffer (pH 7.0), 1 mM EDTA, and 1 mM DTT for determination of 5'D-I activity.

The degree of selenium deficiency in the rats was determined by the decrease in hepatic GPx activity. GPx activity was determined from the oxidation of NADPH in the presence of 0.35 mM t-butyl hydroperoxide monitored spectrophotometrically at 340 nm (15). Samples were run in duplicate and results were expressed as nmol NADPH oxidized/min per mg protein. Hepatic GPx activities in intact, selenium-replete and thyroidectomized, T₄ replaced, selenium-replete rats were 59±58 nmol NADPH oxidized/min per mg protein (n = 9) and 90±53 nmol NADPH oxidized/min per mg protein (n = 12), respectively.

Type I iodothyronine 5′-deiodinase activity was accounted for by the release of radiodiodide from 10 μM [131I]T₃ in the presence of 20 mM DTT (5'D-I) (16). Samples were run in duplicate and results were expressed as units/mg protein; 1 unit of 5'D-I enzyme activity represents the release of 1 pmol radiodiodide/min per mg protein at 37°C.

Hepatic type I iodothyronine 5′-deiodinase activities in intact, selenium-replete and thyroidectomized, T₄ replaced, selenium-replet rats were 224±15 U/mg protein (n = 12) and 1142±8 U/mg protein (n = 9), respectively.

Serum TSH was measured in duplicate by RIA using materials obtained from the National Pituitary Agency, National Institutes of Health (Bethesda, MD). Serum T₄ and T₃ concentrations were determined in duplicate by species-adapted specific RIAs.

Selenium was quantified by measuring the 162 KeV gamma ray produced during the decay of radioactive ⁷⁷Se after irradiation of the sample at a neutron flux. The sensitivity was 0.05 ppm (Research Reactor Facility, University of Missouri–Columbia, Columbia, MO) (17).

Protein was measured by the method of Bradford (18).

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1. Abbreviations used in this paper: BW, body weight; GPx, glutathione peroxidase activity.
and thyroidal 5′D-I appears to be a selenoprotein (7), the near normal serum T₃ concentrations found in intact, selenium-deficient animals raised the possibility that thyroidal 5′D-I was unaffected by selenium deficiency. Thus, we determined the effects of selenium deficiency on thyroidal selenium content (Fig. 2) and on 5′D-I activity in the thyroid (Fig. 3). Rats fed the selenium-deficient diet had a >97% fall in selenium content in the liver and a corresponding >93% decrease in liver 5′D-I activity. However, in the thyroid, the selenium-deficient diet resulted in only a modest 27% decrease in selenium content, and paradoxically, the 5′D-I activity was increased by 25% (P < 0.05).

**Effects of selenium deficiency on intrathyroidal metabolism of 131I.** To evaluate the influence of altered selenium intake on intrathyroidal iodine metabolism, we determined the effects of selenium deficiency on the thyroid gland’s ability to concentrate and organify iodine. Thyroid uptake of 131I was unaffected by selenium intake, and there were no differences in the synthesis of the 131I-labeled iodotyrosines (MIT and DIT) or iodothyronines (T₄ and T₃) between selenium-deficient and selenium-supplemented rats (Table I).

**Discussion**

Controversy surrounds the contribution of the various tissues to T₃ production in the rat. DiStefano (1) estimated that 47% of T₃ originates from both thyroidal secretion and extrathyroidal T₄ to T₃ conversion in liver and kidney, while the remain-

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**Figure 1.** Effect of selenium and thyroidectomy on serum concentrations of T₄, T₃, and TSH in the rat. Rats were fed a defined diet, thyroidectomized, and replaced with T₄ as described in Experimental procedures. Blood was obtained at the time of killing and analyzed for T₄, T₃, and TSH. Intact, nonthyroidectomized rats; T₄ replaced, rats thyroidectomized and replaced with exogenous T₄. *P < 0.05.

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**Figure 2.** Effect of selenium deficiency on the selenium content in the thyroid and liver. Rats were fed a selenium-supplemented (Se+) or selenium-deficient (Se−) diet for 5 wk then were killed. Selenium content was determined as described in Experimental procedures. *P < 0.05.

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**Figure 3.** Effect of selenium deficiency on 5′D-I activity in the thyroid and liver. Rats were fed a selenium-supplemented (Se+) or selenium-deficient (Se−) diet for 5 wk then were killed. 5′D-I activity was assayed as described in Experimental procedures. *P < 0.05.
Intrathyroidal metabolism of $^{131}$I was determined in 5 selenium-supplemented (Se+) and 5 selenium-deficient (Se−) rats as described in Experimental procedures. Results are expressed as mean±SE.

<table>
<thead>
<tr>
<th>% Uptake</th>
<th>Se+</th>
<th>Se−</th>
</tr>
</thead>
<tbody>
<tr>
<td>% MIT</td>
<td>27.4±0.6</td>
<td>27.0±2.2</td>
</tr>
<tr>
<td>% DIT</td>
<td>41.4±1.6</td>
<td>36.9±1.5</td>
</tr>
<tr>
<td>MIT/DIT</td>
<td>0.74±0.08</td>
<td>0.67±0.03</td>
</tr>
<tr>
<td>% T$_3$</td>
<td>1.0±0.3</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>% T$_4$</td>
<td>4.1±0.5</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>T$_3$/T$_4$</td>
<td>0.33±0.06</td>
<td>0.25±0.06</td>
</tr>
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Intrathyroidal iodine metabolism was also unaffected by selenium deficiency, whereas inconsistent results have been reported on the effects of selenium deficiency on intrathyroidal metabolism by others. Goldstein et al. observed a decrease in PB$^{131}$I after in vitro incubation of thyroid glands from selenium-deficient rats with $^{131}$I (27). Arthur et al. (25) found a decrease in both T$_4$ and T$_3$ content in the thyroid gland from selenium-deficient rats, while Meinhold et al. (28) found no change in T$_4$ and T$_3$ content in the thyroid from rats fed a selenium-deficient diet. Taken together, these data suggest that secretion of de novo synthesized T$_3$ is unaffected by selenium deficiency. It is generally assumed that 70–80% of the circulating T$_3$ concentrations (29, 30) is derived from T$_4$ to T$_3$ conversion in humans and similar, albeit, more variable estimates have been made for the rat and that the liver, kidney, and thyroid contain nearly all the 5'D-I activity in the body (6, 30). Since extrathyroidal 5'D-I contributes 10–25% of the T$_3$ production (see above), then more than 50% of the T$_3$ in the thyroidal effluent is likely to be derived from intrathyroidal T$_4$ to T$_3$ conversion of T$_4$ liberated from thyroglobulin.

The finding that the majority of T$_3$ in the rat derives from the thyroid provides a potential explanation for the apparently discordance between the observed $K_m$ for 5'D-I and the concentration of T$_4$ available to extrathyroidal tissues. In vitro estimates of the $K_m$ for T$_4$ for 5'D-I range between 0.5 and 1 μM (6, 30), while the T$_4$ available to the tissues (“free hormone”) is 3–4 orders of magnitude less, indicating that catalysis by 5'D-I in peripheral tissues is very inefficient. However, intracellular T$_4$ levels in the thyroid would be expected to be much greater than those in the circulation, and the $K_m$ for T$_4$ of 5'D-I may reflect the substrate available in the thyroid rather than that in the circulation.

In conclusion, the current study demonstrates that the thyroid gland is a major source of circulating T$_3$ in rats, accounting for approximately 55% of total T$_3$ production. The contri-
duction of intrathyroidal T₄ to T₃ conversion to T₃ homeostasis appears to be important, but the exact contribution remains to be determined.

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