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

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Abstract

In rats, the relative 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. Lauber 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 insufi-
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 Hepes 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 Hepes 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-buty1 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 598±58 nmol NADPH oxidized/min per mg protein (n = 9) and 906±53 nmol NADPH oxidized/min per mg protein (n = 12), respectively.

Type 1 iodothyronine 5'-deiodinase activity was determined by the release of radioiodide from 10 μM [³²I]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 radioiodine/min per mg protein at 37°C. Hepatic type 1 iodothyronine 5'-deiodinase activities in intact, selenium-replete and thyroidectomized, T₄ replaced, selenium-replete rats were 224±15 U/mg protein (n = 12) and 114±8 U/mg protein (n = 9), respectively.

**Serum TSH, serum T₄, T₃, and TSH concentrations in T₄ replaced, thyroidectomized rats.** During the 5-wk experimental period, body weights increased from 55 to 330 g in the euthyroid rats (intact) (n = 17) and from 55 to 289 g in the thyroidectomized rats replaced with T₄ (T₄ replaced) (n = 18), indicating that hormone replacement was almost complete in the thyroidectomized animals. No differences in growth were observed between the selenium-deficient and selenium-supplemented rats. Selenium deficiency resulted in a 97% decrease in hepatic GPx activity and a parallel 93% decrease in hepatic 5'D-I activity in both the intact and T₄-replaced rats indicating that the animals were selenium deficient. As shown in Fig. 1, in selenium-supplemented rats, serum T₄ concentrations were similar in the intact and T₄ replaced groups (A). Selenium deficiency resulted in the expected increase in the serum T₄ concentrations in both the intact and T₄ replaced groups (P < 0.05, A vs. B).

Although the serum T₄ concentrations were identical in the selenium-supplemented intact and T₄ replaced rats, serum T₃ concentrations were not normalized and T₃ concentrations in the T₄ replaced rats remained 55% lower than those in the intact group (Fig. 1 C). Selenium deficiency did not significantly affect serum T₃ values in the intact rats (1.23 nM±0.14 vs. 1.11±0.10, selenium-supplemented vs. selenium-deficient rats).
rats, respectively) but decreased the serum T₃ concentrations by 20% in the T₄ replaced group (C and D) (0.55±0.03 nM vs. 0.44±0.02 nM, P < 0.05 selenium-supplemented vs. selenium-deficient rats, respectively).

In both selenium-supplemented and selenium-deficient thyroidecomized rats, T₄ replacement did not normalize the serum TSH concentrations, and they remained 2 to 3 times higher than those observed in the intact rats (Fig. 1, E and F). Selenium deficiency did not affect serum TSH concentrations in the intact rats.

Effect of selenium deficiency on selenium content and 5'D-I activity in the thyroid. The failure of T₄ replacement to normalize serum T₃ concentrations in the selenium-supplemented rats, despite the availability of normal T₄ levels to the 5'D-I containing tissues, suggested that the thyroid gland may contribute up to 55% of the T₃ found in the circulation. Similarly, the thyroid appeared to contribute as much as 60% of the circulating T₃ in the selenium-deficient rats, animals that lack the selenoprotein 5'D-I in the liver and kidney. Since earlier work suggested that intrathyroidal T₄ to T₃ conversion was a major contributor to the T₃ found in the dog thyroid effluent (4, 5), 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 1).

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-

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.

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 1. Effect of Selenium on Intrathyroidal $^{131}$I Metabolism

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<thead>
<tr>
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<th>Se+</th>
<th>Se−</th>
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<tbody>
<tr>
<td>% Uptake</td>
<td>9.4±1.3</td>
<td>12.7±3.2</td>
</tr>
<tr>
<td>% MIT</td>
<td>27.4±0.6</td>
<td>27.0±2.2</td>
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<tr>
<td>% DIT</td>
<td>41.4±1.6</td>
<td>36.9±1.5</td>
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<tr>
<td>MIT/DIT</td>
<td>0.74±0.08</td>
<td>0.67±0.03</td>
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<tr>
<td>% T3</td>
<td>1.0±0.3</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>% T4</td>
<td>4.1±0.5</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>T3/T4</td>
<td>0.33±0.06</td>
<td>0.25±0.06</td>
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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 $^{131}$I uptake after in vitro incubation of thyroid glands from selenium-deficient rats with $^{131}$I (27). Arthur et al. (25) found a decrease in both T3 and T4 content in the thyroid gland from selenium-deficient rats, while Meinhold et al. (28) found no change in T4 and T3 content in the thyroid from rats fed a selenium-deficient diet. Taken together, these data suggest that secretion of de novo synthesized T3 is unaffected by selenium deficiency. It is generally assumed that 70–80% of the circulating T3 concentrations (29, 30) is derived from T3, to T3 conversion in human and similar, albeit, more variable estimates have been made for the rat and that the liver, kidney, and thyroid contain nearly all the $^{131}$I activity in the body (6, 30). Since extrathyroidal $^{131}$I contributes 10–25% of the T3 production (see above), then more than 50% of the T3 in the thyroidal effluent is likely to be derived from extrathyroidal T4 to T3 conversion of T4 liberated from thyroglobulin.

The finding that the majority of T3 in the rat derives from the thyroid provides a potential explanation for the apparent discordance between the observed Km of 5'D-I and the concentration of T4 available to extrathyroidal tissues. In vitro estimates of the Km for T4 for 5'D-I range between 0.5 and 1 μM (6, 30), while the T4 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 T4 levels in the thyroid would be expected to be much greater than those in the circulation, and the Km for T4 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 T3 in rats, accounting for approximately 55% of total T3 production. The contrib-
bution 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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References


