The formation of tri-iodothyronine and reverse tri-iodothyronine from thyroxine in isolated rat renal tubules

P. HEYMA, R. G. LARKINS(1), J. R. STOCKIGT(2) AND D. G. CAMPBELL

Department of Medicine, University of Melbourne, Department of Biochemistry, Royal Melbourne Hospital, Victoria, Australia; Department of Medicine, Repatriation General Hospital, Heidelberg, Victoria, and Ewen Downie Metabolic Unit, Alfred Hospital, Victoria, Australia

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Summary

1. Conversion of thyroxine into tri-iodothyronine and reverse tri-iodothyronine in intact cells was studied with isolated renal tubules prepared by collagenase digestion.
2. Conversion of thyroxine into tri-iodothyronine and reverse tri-iodothyronine increased progressively for at least 90 min.
3. Studies of tri-iodothyronine production from increasing amounts of thyroxine revealed that the thyroxine to tri-iodothyronine conversion is saturable.
4. Iodide and carbimazole had no effect on the thyroxine to tri-iodothyronine conversion.
5. 6-Propyl-2-thiouracil had a direct non-competitive inhibitory effect on the conversion of thyroxine into tri-iodothyronine with a 75% inhibition of the conversion at a propylthiouracil concentration within the therapeutic range in vivo. Propylthiouracil also inhibited the net formation of reverse tri-iodothyronine from thyroxine at a similar propylthiouracil concentration, as well as inhibiting the subsequent degradation of reverse tri-iodothyronine.

Key words: carbimazole, iodide, non-competitive inhibition, 6-propyl-2-thiouracil, renal tubules, reverse iodothyronine, thyroxine, tri-iodothyronine.

Abbreviations: T4, thyroxine; T3, tri-iodothyronine; rT3, reverse tri-iodothyronine.

Correspondence: Dr P. Heyma, Department of Medicine, Royal Melbourne Hospital, Melbourne, Victoria, 3050, Australia.

Introduction

Since the first observations of the extra-thyroidal conversions, thyroxine (T4) into tri-iodothyronine (T3) (Gross & Leblond, 1951; Gross & Pitt-Rivers, 1952), there has been a growing awareness of the importance of this conversion in the regulation of thyroid hormone action. More recently, thyroxine has been shown to be de-iodinated peripherally to reverse tri-iodothyronine (rT3) (Chopra, 1974), as well as to T3. Although rT3 is apparently biologically inactive, this alternative metabolic pathway is potentially of great importance in the overall regulation of iodothyronine metabolism. Several systems in vitro have been devised for the study of T4 de-iodination, such as liver homogenates (Visser, van der Does-TobC, Docter & Hennemann, 1975), liver and kidney homogenates (Chopra, 1977), kidney slices (Albright, Larson & Tust, 1954) and kidney cells in culture (Sterling, Brenner & Saldahna, 1973), but these systems may not be physiologically ideal for studying these reactions. Isolated cells that appear to have intact membranes, intracellular structure and biochemical systems may be more physiological than tissue homogenates. Tissue slices have the disadvantage that uneven perfusion with medium may produce cellular necrosis (Elliot & Libet, 1942), and cells in culture are less likely to retain specific organ function than suspensions of freshly dispersed cells (Weigand, Müller, Urban & Schreiber, 1971; East, Louis & Hoffenberg, 1973). The ease of preparation of isolated renal tubules, their long-term viability and the demonstration of the role of the kidney in thyroxine de-iodination
(Albright et al., 1954; Larson, Tomita & Albright, 1955) make isolated renal tubules useful for studying this reaction. We describe such a system and have studied the kinetics of the conversion of T4 into T3 and also the effects of iodide, propylthiouracil and carbimazole upon this system.

Materials and methods

Preparation of the renal tubules

Sprague-Dawley rats (100 g) were fed on Purina Dog Chow ad libitum and allowed free access to water. Rats were anaesthetized with ether and kidneys excised and washed with Hanks buffer, pH 7.2 (Hanks & Wallace, 1949). The renal tubules were isolated by a method based on that of Burg & Orloff (1962). The kidneys were cut into small pieces and placed in 10 ml of Hanks buffer containing 30 mg of collagenase (144 unit/mg, CLS 46070P; Worthington Biochemical Corporation, New Jersey, U.S.A.) and 10 mg of human serum albumin (Commonwealth Serum Laboratories, Victoria, Australia). The suspension was shaken vigorously at 37°C for 15 min, then filtered through 8 μm nylon gauze and centrifuged at 70 g at 4°C for 1 min (MSE 4L centrifuge). The supernatant was decanted and the tubules were washed three times in phosphate buffer, pH 7.2 (Dulbecco & Vogt, 1954), containing 0.1% human serum albumin. The suspension of tubules was then made up to 15 ml with Dulbecco buffer, and divided into 1.0 ml aliquots while being gently stirred. In each experiment, one aliquot was reserved for determination of protein content (Meulemans, 1960).

Incubation conditions

The tubule aliquots were kept at 4°C while 0.64 nmol of thyroxine (Sigma Chemical Co., Mo., U.S.A.), purified according to the method of Kjeld, Kuku, Diamond, Fraser, Joplin & Mashiter (1979), was added. The vials were then shaken gently for 20 min at 37°C. The contents of each vial were centrifuged at 1500 g at 4°C and the supernatants decanted. The tubules were then extracted with 0.5 ml of ethanol (Merck, Darmstadt, Germany). By using radioactively labelled tri-iodothyronine, the efficiency of extraction was found to be 94.4 ± 3% (SD). Extracts and supernatants were diluted 1:5 with charcoal-treated (T4- and T3-free) serum before assay. The charcoal-treated serum had at least 99% of the T3 and T4 removed. Control samples at zero time were included with every incubation and used to correct for any cross-reaction of T4 in the T3 assay. Measurements were performed in duplicate in each incubation. Comparisons were made by the unpaired t-test.

Tests of renal tubule viability

Carbon dioxide production from glucose was studied by using the method of Shain & Barnea (1971). Amino acid incorporation into protein was performed by the method of Mans & Novelli (1960). The viability of the tubules was also assessed by their ability to exclude eosin dye under the light microscope.

Measurement of T3, T4 and reverse tri-iodothyronine (rT3)

The T3 radioimmunoassay used a high specific radioactivity tracer (3.1 μCi/μmol) (Weeke & Orskov, 1973). A portion (50 μl) of tri-iodothyronine standards (Sigma), diluted extracts or supernatants was incubated for 16 h at room temperature with 200 μl of tracer (15 000 d.p.m.) and 100 μl of tri-iodothyronine antiserum (Calbiochem, Calif., U.S.A.) diluted 1:200. The assay was performed in a final volume of 1.0 ml of phosphate buffer (0.07 mol/l), pH 7.4, containing merthiolate (0.25 mmol/l) and gelatin (0.1 g/100 ml). Separation of free and antibody-bound tri-iodothyronine was carried out by using dextran-coated charcoal, 1.5 g/100 ml of charcoal (M.C.B., Ohio, U.S.A.) and 0.15 g of dextran T70/ml (Pharmacia, Sweden). Supernatants were counted for radioactivity in a Packard Auto Gamma Scintillation Spectrometer. The inter-assay coefficient of variation was less than 10% and the sensitivity 0.01 pmol/tube. Cross-reactivity of the purified T4 in the T3 radioimmunoassay was 0.05%. The T4 radioimmunoassay used a commercial kit (The Radiochemical Centre, Amersham, Bucks., U.K.). The interassay coefficient of variation was less than 10%. Reverse tri-iodothyronine (rT3) was measured by radioimmunoassay with a rabbit antiserum to LrT3 at a final dilution of 1:24000, giving an assay sensitivity of 0.01 pmol/tube with double-antibody separation. 125I-labelled rT3 with a specific radioactivity of 1.5–2.3 μCi/μmol was prepared by the method of Burger & Ingbar (1974). Extracts, supernatants and standards were diluted in charcoal-treated, T4- and T3-free serum, with 8-anilinonaphthalenesulphonic acid (1.6 mmol/l) added to inhibit binding of hormone to
plasma proteins. Samples and standards were assayed in 0.1 ml of serum in a final volume of 1.0 ml of barbitone buffer (60 mmol/l, pH 8.6) containing 2 g of gelatid. The antiserum showed 0.1% cross-reactivity with purified T4. All rT3 results were expressed in terms of L-rT3 standard from Henning, Berlin.

Time course of thyroid hormone degradation and conversion

Aliquots (1 ml) of renal tubules were incubated with 0.45 nmol of T4, or 5.3 pmol of T3 or 15.4 pmol of rT3 with or without 4 pmol of 6-propyl-2-thiouracil (Sigma) for intervals up to 60 min. T4, T3 and rT3 assays were performed on tubule extracts and supernatants. Each sample at each time was assayed in duplicate.

Aliquots (1 ml) of renal tubules were incubated with 0.64 nmol of T4 for intervals up to 90 min. T3 and rT3 assays were performed on extracts and supernatants. Each sample at each time was assayed in duplicate.

T4 to T3 and rT3 conversion

The effects of increasing amounts of (i) renal tubules, (ii) thyroxine, (iii) potassium iodide, (iv) propylthiouracil, (v) carbimazole and (vi) increasing amounts of thyroxine in the presence of propylthiouracil (4 µmol/l) were also studied. In all cases incubations proceeded for 20 min, and T3 and rT3 assays were performed as outlined above.

Results

The renal tubule preparations produced carbon dioxide from glucose at a constant rate over a 4 h period. Amino acid incorporation into protein also progressed linearly over a similar time period; 60–70% of the tubules were still capable of excluding eosin dye after 8 h incubation at 37°C.

The degradation studies (Table 1) showed that there was a slight decline in total recoverable T4

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Hormone recovery (%) of initial</th>
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<tr>
<td></td>
<td>T4</td>
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<tr>
<td>0</td>
<td>100</td>
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<td>5</td>
<td>95</td>
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<td>90</td>
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<td>30</td>
<td>90</td>
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<td>60</td>
<td>88</td>
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![Fig. 1. Time course of production of (a) tri-iodothyronine (T3) and (b) reverse tri-iodothyronine (rT3) from thyroxine (T4) by isolated renal tubules. Measurements for each time point were performed in duplicate, and duplicate values are shown. Total T3 and rT3 values were obtained by the sum of the mean of extract and supernatant values. ○, Total T3 or rT3 produced; ■, T3 or rT3 in cell extracts; ▲, T3 or rT3 in supernatants; ◆, total T3 produced by heat-inactivated cells (60 min point only).](image-url)
FIG. 2. Formation of (a) tri-iodothyronine (T3) and (b) reverse tri-iodothyronine (rT3) from increasing amounts of thyroxine (T4). (a) The combined mean results (±SEM) of three experiments are shown, each performed in duplicate. (b) The results of one experiment; the determinations for each point were performed in duplicate, and duplicate values are shown.

FIG. 3. Effect of $4 \times 10^{-6}$ mol/l propylthiouracil (PTU) on (a) tri-iodothyronine (T3) production and (b) reverse tri-iodothyronine (rT3) production from increasing amounts of thyroxine (T4). ● No PTU; ■ with $4 \times 10^{-6}$ mol/l PTU. Each graph represents the results of one experiment; the determinations for each point were performed in duplicate, and duplicate values are shown.

**TABLE 2. Effect of propylthiouracil and carbimazole on tri-iodothyronine (T3) and reverse tri-iodothyronine (rT3) production from thyroxine (T4) by isolated rat renal tubules**

Results for T3 formation are the combined results from three experiments, providing eight control values and four values for each propylthiouracil (PTU) and carbimazole concentration. For rT3, duplicate values from a single experiment are shown. All results are expressed as a percentage of the mean value for the appropriate control replicates. n.s., Not significant.

<table>
<thead>
<tr>
<th>Conc. (mol/l)</th>
<th>Total recoverable T4 (% of control)</th>
<th>T3 formed (% of mean of control ± SEM)</th>
<th>P (compared with control)</th>
<th>rT3 formed (% of mean of control)</th>
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<tr>
<td>Propylthiouracil</td>
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<tr>
<td>4 × 10^{-8}</td>
<td>99,90</td>
<td>85 ± 16</td>
<td>n.s.</td>
<td>55,60</td>
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<tr>
<td>4 × 10^{-7}</td>
<td>96,97</td>
<td>45 ± 6</td>
<td>&lt;0.01</td>
<td>55,65</td>
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<tr>
<td>4 × 10^{-6}</td>
<td>93,92</td>
<td>24 ± 8</td>
<td>&lt;0.001</td>
<td>50,60</td>
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<td>96,98</td>
<td>26 ± 6</td>
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<tr>
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<td>106,90</td>
<td>34 ± 7</td>
<td>&lt;0.001</td>
<td>28,34</td>
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<tr>
<td>4 × 10^{-3}</td>
<td>101,97</td>
<td>43 ± 5</td>
<td>&lt;0.01</td>
<td>14,25</td>
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<tr>
<td>Carbimazole</td>
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<tr>
<td>5-4 × 10^{-7}</td>
<td></td>
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<td>5 × 10^{-6}</td>
<td>91 ± 5</td>
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<td>5 × 10^{-5}</td>
<td>109 ± 4</td>
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<tr>
<td>5 × 10^{-4}</td>
<td>109 ± 5</td>
<td>n.s.</td>
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<tr>
<td>3 × 10^{-3}</td>
<td>99 ± 3</td>
<td>n.s.</td>
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<td></td>
<td>100 ± 7</td>
<td>n.s.</td>
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and no significant decline in total recoverable T3 after incubation with renal tubules for 60 min. However, reverse tri-iodothyronine (rT3) was rapidly degraded by the renal tubules, but this was inhibited by propylthiouracil. The net formation of T3 and rT3 from T4 increased progressively for at least 90 min with T3 and rT3 appearing in the supernatant between 10 and 20 min (Fig. 1). The conversion did not occur in heat-inactivated cells.

The production of T3 from a given amount of T4 was directly proportional to the amount of renal tubules present (as mg of protein) up to a concentration of 5-6 mg of protein/tube. Formation of T3 and rT3 from increasing amounts of thyroxine showed a plateau of T3 and rT3 production at thyroxine concentrations of 9 μmol/l and 14 μmol/l respectively (Fig. 2). Potassium iodide in concentrations from 10-6 mol/l to 10-1 mol/l had no effect on T3 production from T4; however, a significant inhibitory effect of propylthiouracil on both T3 and rT3 formation from T4 was observed (Table 2). Carbimazole had no effect on the conversion (Table 2). The inhibition by propylthiouracil of both T3 and rT3 formation from increasing amounts of T4 was not overcome by T4 concentrations up to 14 μmol/l (Fig. 3).

Discussion

Early work on thyroxine to tri-iodothyronine conversion in vitro used radioactively labelled thyroxine, which necessitated correction for spontaneous de-iodination of thyroxine to tri-iodothyronine (Albright et al., 1954). These problems were overcome in our system by using purified non-radioactive thyroxine and sensitive radioimmunoassays for tri-iodothyronine and reverse tri-iodothyronine.

Apart from the work of Sterling et al. (1973), who used monolayer cultures of human kidney cells, isolated kidney cells have not been used to study the conversion of thyroxine into tri-iodothyronine. Liver cells have been used (Hesch, Bruner & Solig, 1975), but they appear to retain their biochemical viability for only 1 h (Berry & Friend, 1969), which limits their potential usefulness in long-term studies. Rat renal tubules are viable for long periods and provide an ideal system for studying thyroid hormone conversion in vitro.

Both T3 and T4 are stable in the renal tubule system for over 1 h, but reverse tri-iodothyronine (rT3) is rapidly degraded by the renal tubules and has a half-life of approximately 10 min in the system. This is consistent with a previous report showing that rT3 is very unstable in rat liver homogenate preparations (Hüffner, Grussendorf & Ntokalou, 1977). Isolated renal tubules are capable of immediate production of T3 and rT3 with no discernible plateau by 90 min. In view of the short half-life of rT3 it is apparent that real rT3 production in the renal tubule system is greater than that observed and that under the experimental conditions described exceeds T3 production. rT3 production from T4 has been observed in rat liver homogenate (Hüffner et al., 1977) but at a somewhat lower rate than in the renal tubule preparation.

We have shown that production of total T3 from increasing amounts of T4 begins to plateau at a T4 concentration of approximately 9 μmol/l, indicating that the converting system can be saturated. Sterling et al. (1973) studied T3 production from T4 up to a maximum T4 concentration of only 5 μmol/l and were unable to show any plateau; this apparent difference probably arises from the differences in T4 concentration ranges studied.

As iodide is a product of T4 de-iodination, we studied the possibility that iodide might inhibit conversion of T4 into T3, but failed to find any effect from extracellular inorganic iodide. The extrathyroidal effects of propylthiouracil have been known for over a decade (Escobar del Rey & Morreale de Escobar, 1961) and Braverman & Ingbar (1962) found inhibition of T4 to T3 conversion by propylthiouracil in kidney slices, which we can confirm by showing a significant inhibition of T4 to T3 conversion by propylthiouracil at concentrations of 10-7 mol/l and above. Propylthiouracil does not affect uptake of T4 into the cells and the inhibitory effect is not due to T4 depletion, as the total recoverable T4 was not reduced by propylthiouracil. The concentration of propylthiouracil where inhibition was observed correlates well with quoted therapeutic concentrations of approximately 10-5 mol/l (McMurray, Gilliland, Ratliff & Bourland, 1975). Propylthiouracil inhibition was shown to be specific in our system, as another thioamide (carbimazole) that acts directly on the thyroid had no effect on the conversions. We have shown that propylthiouracil decreases net rT3 production from thyroxine, and that propylthiouracil reduces degradation of rT3 in intact cells. Westgren, Melander, Wahlin & Lindgren (1977) demonstrated an increase in rT3 concentrations after propylthiouracil treatment in vivo, whereas we find that in vitro propylthiouracil inhibits both formation and degradation.
of rT3. In our system the net effect was a decrease of rT3 concentration, but in vivo the effect on rT3 degradation appears to predominate.

The inhibition by propylthiouracil of both T4 to T3 to rT3 conversion is non-competitive, as it is not affected by increasing amounts of T4. This is in agreement with the work of Chopra (1977), who showed that propylthiouracil is a non-competitive inhibitor of T4 to T3 conversion in a rat liver-homogenate system.

Acknowledgment

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References


