The effect of iodothyronines on the conversion of thyroxine into 3,3',5-tri-iodothyronine in isolated rat renal tubules

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Isolated rat renal tubules prepared by collagenase digestion were used to study the effects of 3,3',5'-tri-iodothyronine ('reverse T_3 ', rT_3) and other iodothyronines on the formation of 3,3',5-tri-iodothyronine (T_3) from thyroxine (T_4). rT_3 inhibited the conversion with a dose response over the concentration range 1.5 nM–1.5 μ M. The inhibition was competitive in nature. Both 3,3'-di-iodothyronine and 3',5'-di-iodothyronine also inhibited the production of T_3 from T_4 in isolated rat renal tubules, but tetraiodothyroacetic acid and 3,5-di-iodothyronine were found to have no effect. These experiments demonstrate in an intact cell system that some naturally occurring iodothyronines have significant effects on T_4 deiodination.

The idea that structurally similar compounds to T_4 may inhibit its biological activity was first suggested by Woolley in 1946 (Woolley, 1946). It has been demonstrated *in vivo* that T_4 is converted in part into T_3 , biologically a much more active compound. The effect of iodothyronines on T_4 deiodination was first studied in kidney slices by Larson & Albright (1961) with 131 I-labelled T_4 . Since then many systems have been devised for the study of T_4 deiodination *in vitro* and the effects of various compounds on the reaction. The development of sensitive radioimmunoassays for the quantification of thyroid hormones has facilitated the experimental procedures involved in studying T_4 deiodination as well as improving sensitivity and accuracy of the results obtained. Chopra (1977) studied the effects of iodothyronines on T_4 deiodination in rat liver homogenates by using radioimmunoassay techniques. The development of a rat renal-tubule system (Heyma *et al.*, 1978) has enabled us to study the effects of iodothyronines on T_4 deiodination to T_3 in an intact-cell preparation. The results obtained were compared with those obtained in rat renal homogenates.

Materials and methods

Incubation procedure

Renal tubules were prepared as described previously (Heyma *et al.*, 1978). In each experiment

Abbreviations used: T_4 , thyroxine; T_3 , 3,3',5-tri-iodothyronine.

renal tubules from two kidneys were made up to 15 ml with phosphate buffer, pH 7.2 (Dulbecco & Vogt, 1954) containing 1 g of human serum albumin/litre and 5.6 mM-D-glucose. The suspension was divided into 1.0 ml portions while being gently stirred, one portion being reserved for determination of protein content by the method of Meulemans (1960). The tubule portions were kept at 4°C while 0.64 nmol of T_4 purified by the method of Kjeld *et al.* (1975) was added to each vial. The iodothyronines investigated were; 3,3',5'-tri-iodothyronine ('reverse T_3 '), 3,3'-di-iodothyronine (Henning, Berlin, Germany) and 3,5-di-iodothyronine (Sigma Chemical Co., St. Louis, MO, U.S.A.). Tetraiodothyroacetic acid (Sigma) was also studied. The iodothyronines were all at least 99% pure and had low cross-reactivity in the T_3 radioimmunoassay. The iodothyronine under study was added to the vials to give final concentrations ranging from 1 nM to 1 μ M. Incubations at each concentration were performed in quadruplicate and vials with only T_4 and vehicle added were included in each experiment in quadruplicate as controls. The vials were then shaken gently at 37°C for 20 min. The contents of each vial were centrifuged and tubules were extracted with ethanol and assayed by radioimmunoassay as described before (Heyma *et al.*, 1978). Samples with T_4 added at zero time were also included in every incubation and used to correct for any cross-reactivity of T_4 in the T_3 assay. Zero-time controls (controls in which T_4 was added to the tubules after incubation) containing the iodothyronine under study

were included in the appropriate experiment to correct for any cross-reactivity of the iodothyronine in the T_3 assay. The cross-reactivity of the purified T_4 in the T_3 radioimmunoassay was 0.05% and this cross-reactivity only became significant at T_4 concentrations of $6\mu\text{M}$ and above in which T_3 values of zero-time controls or blanks accounted for about 13% of T_3 formed. The results of each experiment were expressed as a percentage of the mean of the control vials (T_4 present, no iodothyronine).

Studies with increasing substrate concentration

Portions of renal-tubule suspension were incubated with increasing amounts of purified T_4 to a final T_4 concentration of $13\mu\text{M}$. An identical set of renal tubules were incubated with the same amounts of T_4 but with each vial containing $3,3',5'$ -tri-iodothyronine at a concentration of $0.154\mu\text{M}$ - $3,3',5'$ -tri-iodothyronine. Incubations at each T_4 concentration were performed in duplicate and zero-time samples at each T_4 concentration were also included. The incubation proceeded for 20 min at 37°C and the extraction and T_3 radioimmunoassay procedures were carried out as described previously.

Preparation of the homogenate

Two sliced rat kidneys were homogenized at 4°C in 3 ml of phosphate buffer (Dulbecco & Vogt, 1954) containing glucose and albumin in a hand-held homogenizer with a tight-fitting Teflon piston (Thomas, Philadelphia, PA, U.S.A.). Twelve full strokes were found to be optimal for homogenization. The homogenate was made up to 15 ml with the phosphate buffer containing glucose and albumin and divided into 0.5 ml portions. Studies were performed as described above with the whole-cell system, and the extraction and T_3 radioimmunoassay procedures were performed as outlined above.

Results

In the experiments with the intact tubules, a mean

of 1.0 ± 0.3 ($\pm$ s.e.m.) pmol of T_3 /mg of protein per 20 min was generated from 0.64 nmol of purified T_4 in the control tubes from different experiments. $3,3',5'$ -Tri-iodothyronine significantly inhibited the formation of T_3 from T_4 by the renal tubules at a concentration of 15 nM ($P < 0.02$) and increasing inhibition was observed with increasing concentrations of $3,3',5'$ -tri-iodothyronine up to $1.5\mu\text{M}$ with 47% inhibition at this concentration ($P < 0.001$) (Table 1). The inhibition by $0.15\mu\text{M}$ - $3,3',5'$ -tri-iodothyronine formation was overcome by increasing T_4 concentrations (Fig. 1). Similar results were obtained with the kidney homogenate studies and the Lineweaver-Burk plots of the data confirmed that the inhibition of T_3 formation from T_4 by $3,3',5'$ -tri-iodothyronine is competitive in nature (Fig. 2), i.e. the V_{max} for the reaction with and without $3,3',5'$ -tri-iodothyronine was the same (0.5 pmol of T_3 /mg of protein per 20 min), but the K_m for the reaction was different in the presence of $3,3',5'$ -tri-iodothyronine ($1.25\mu\text{M}$) and absence of $3,3',5'$ -tri-iodothyronine ($5\mu\text{M}$). Of the other iodothyro-

Fig. 1. Effect of $3,3',5'$ -tri-iodothyronine on T_3 formation from increasing amounts of T_4 by isolated rat renal tubules

The results of one experiment are shown, with duplicates performed at each concentration. Symbols: ●, no $3,3',5'$ -tri-iodothyronine; ○, $+0.15\mu\text{M}$ - $3,3',5'$ -tri-iodothyronine.

Table 1. Effect of $3,3',5'$ -tri-iodothyronine on T_3 formation from T_4 by isolated rat renal tubules

The combined results of two experiments are shown, with quadruplicates performed at each concentration in each experiment. Abbreviation used: n.s., not significant.

$3,3',5'$ -tri-iodothyronine concn.	T_3 produced (% of mean of controls $\pm$ s.e.m.)	P value compared with controls
0 ($n = 8$)	100 ± 4	—
0.15 nM ($n = 8$)	97 ± 11	n.s.
1.5 nM ($n = 8$)	88 ± 4	n.s.
15 nM ($n = 8$)	84 ± 2	< 0.02
$0.15\mu\text{M}$ ($n = 8$)	69 ± 8	< 0.01
$1.5\mu\text{M}$ ($n = 8$)	63 ± 2	< 0.001

Fig. 2. Effect of 3,3',5'-tri-iodothyronine on T_3 formation from increasing amounts of T_4 by rat kidney homogenates (a) The results of one experiment are shown, with duplicates at each T_4 concentration. Symbols: ●, no 3,3',5'-tri-iodothyronine; ○, +0.15 μM -3,3',5'-tri-iodothyronine. (b) shows a Lineweaver-Burk plot of the data.

Fig. 3. Effect of tetraiodothyroacetic acid (a), 3,5-(b), 3',5'-(c) and 3,3'-di-iodothyronine (d) on T_3 production from T_4 by isolated rat renal tubules

The results of four separate experiments are shown, with quadruplicates performed at each concentration in each experiment. Significances are denoted as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

nines studied, 3,3'-di-iodothyronine and 3',5'-di-iodothyronine inhibited T_3 production from T_4 by 50% at concentrations of 1 μM and 2 μM respectively. Tetraiodothyroacetic acid and 3,5-di-iodothyronine had no effect on the formation of T_3 and T_4 by the tubules (Fig. 3).

Discussion

The regulation of thyroid hormone metabolism in man and animals is as yet poorly understood, but a

large number of studies *in vivo* have suggested that iodothyronine metabolism, particularly the deiodination of T_4 to T_3 and 3,3',5'-tri-iodothyronine is not a random process but under metabolic control. The introduction of radioimmunoassay for 3,3',5'-tri-iodothyronine (Chopra, 1974) showed that it was present in the circulation in significant concentrations. Studies *in vivo* that followed showed a reciprocal relationship between serum concentrations of T_3 and 3,3',5'-tri-iodothyronine in severe illness (Carter *et al.*, 1976), in surgical trauma (Adami *et al.*, 1978), neonatal animals (Chopra *et al.*, 1975) and during starvation (Vagenakis *et al.*, 1975). A possible explanation was that 3,3',5'-tri-iodothyronine might somehow regulate the production of T_3 from the common precursor T_4 . Investigations *in vitro* followed rapidly, most of the studies being performed in homogenate systems (Chopra, 1977; Kaplan & Utiger, 1978).

Larson & Albright (1961) demonstrated the competitive nature of the inhibition of 3,3',5'-tri-iodothyronine of the deiodination of T_4 to T_3 . This study was performed using kidney slices. Studies with rat liver homogenates (Chopra, 1977; Kaplan & Utiger, 1978) and in leucocytes (Woeber, 1978) have also been carried out. The results of the present experiments confirm these previous studies. We observed significant inhibition of T_3 formation from T_4 at 3,3',5'-tri-iodothyronine concentrations of 15 nM and above. The half-life of 3,3',5'-tri-iodothyronine in the renal tubules is of the order of 10 min (Heyma *et al.*, 1978), so the effective concentration of 3,3',5'-tri-iodothyronine in the incubation system would be close to the physiological concentration of about 1 nM (Griffiths *et al.*, 1976; Chopra, 1976). However, because the T_4 concentrations are high and protein concentrations

low compared with estimations *in vivo*, it is not possible to make definite statements regarding the situation *in vivo* from the data *in vitro*. The physiological importance of the 3,3',5'-tri-iodothyronine studies *in vitro* has been questioned (Nicod *et al.*, 1976) and the biological significance of 3,3',5'-tri-iodothyronine still remains unclear. This, however, may not exclude 3,3',5'-tri-iodothyronine from an important role in the regulation of iodothyronine metabolism, but the rapid degradation of 3,3',5'-tri-iodothyronine *in vitro* (Hüfner *et al.*, 1977; Visser *et al.*, 1978; Heyma *et al.*, 1978) and high turnover rate *in vivo* (Dunn & Stanburg, 1958; Chopra, 1976) make studies of this iodothyronine difficult.

The results of the studies with the other iodothyronines are in general agreement with those of Chopra (1977) who studied the effect of iodothyronines in rat liver homogenates. We also found inhibition of T_3 formation from T_4 with 3,3'- and 3',5'-di-iodothyronine and no effect with 3,5-di-iodothyronine. In contrast with that study we found no effect of tetraiodothyroacetic acid on T_3 production from T_4 . This may reflect differences between homogenate and intact-cell systems. Gavin *et al.* (1978) has shown that 3,3'-di-iodothyronine is a major product of the deiodination of both T_3 and 3,3',5'-tri-iodothyronine, and more recent work suggests that 3,5'-di-iodothyronine is an important product of 3,3',5'-tri-iodothyronine deiodination (Geola *et al.*, 1979). The findings that both 3,3'- and 3',5'-di-iodothyronine inhibit the deiodination of T_4 and T_3 *in vitro* suggest that like 3,3',5'-tri-iodothyronine they may be potential modulators of thyroid hormone metabolism. All the compounds that inhibit conversion of T_4 into T_3 *in vitro* share an outer-ring iodine atom and are potential substrates for an outer-ring deiodinase enzyme. The interaction between these compounds provides strong evidence that a single enzyme is involved in the outer-ring deiodination of each of these compounds.

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