Glutathione deficiency and peripheral metabolism of thyroid hormones during dietary cysteine deprivation in rats

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1. For a period of 24 d, young rats received a diet containing 120 g casein/kg or the same basic diet supplemented with 1·93 g cysteine/kg.

2. The thyroxine (T_4) turnover was decreased in rats receiving the cysteine-deficient diet compared with that of rats on the supplemented diet. Moreover, the extrathyroidal T_4 pool and T_4 disposal rate decreased.

3. Cysteine deprivation also decreased the peripheral metabolism of 3,5,3′-triiodothyronine (T_3). The T_3 distribution space, extrathyroidal pool of T_3 and T_3 disposal rate were diminished.

4. In vitro, deiodination of T_4 in liver homogenate assayed with endogenous glutathione (GSH) demonstrated decreased T_3 production rates in the case of cysteine deficiency. This difference was minimized by the addition of GSH in amounts sufficient to saturate the reaction kinetics. In the light of this finding, GSH is probably involved in the promotion of certain thyroidal problems induced by a cysteine-deficient diet.

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine; GSH) is a tripeptide which has many metabolic functions in the liver and kidney (for review, see Sies & Wendel, 1978). For example, GSH is critical for thyroid hormone metabolism as it is the cofactor of the 5′-monodeiodinase (Type I) enzyme which catalyses the deiodination of thyroxine (T_4) to 3,5,3′-triiodothyronine (T_3) (Balsam & Ingbar, 1978; Chopra, 1978; Imai *et al.* 1980; Higueret & Garcin, 1982) and affects T_3 cellular uptake (Higueret & Garcin, 1984).

Nutritional conditions, such as the cysteine and methionine contents of the diet, influence the GSH content in tissues (Tateishi *et al.* 1981; Glazenburg *et al.* 1983; Suberville *et al.* 1987). Recently, we have shown that thyroidal diseases appeared in animals fed on an imbalanced diet which was deficient in either cysteine or cysteine and methionine (Suberville *et al.* 1987). In order to identify the precise mechanisms involved in changes of hormonal status, we have studied some aspects of peripheral metabolism of thyroid hormones during dietary cysteine deprivation. Cysteine depletion was achieved with diets in which the protein source was casein at 120 g/kg. The control diet contained 120 g casein/kg and was supplemented with 1·93 g cysteine/kg which meets the minimum nutritional requirements for sulphur amino acids in the rat (Sowers *et al.* 1972).

METHODS

Animals and diets

Male Wistar rats with an initial weight of 110–120 g were obtained from IFFA Credo (France) and were fed for 24 d on one of the following semi-synthetic diets (g/kg): casein 120, casein 120 + L-cysteine 1·93 (Suberville *et al.* 1987). Animals were housed in an air-conditioned room with a mean temperature of $21 \pm 1°$, were weighed daily and the food

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intake calculated. Under these experimental conditions, as we have already shown (Suberville *et al.* 1987), the food intakes of the two groups of animals are similar.

At the end of the experimental period the rats were killed by decapitation at 09.00 hours and the livers rapidly excised. A portion was immediately used for GSH analysis, the remainder was frozen in liquid nitrogen and stored at $-80°$ for subsequent analysis.

In vivo metabolism of T_4 and T_3

Turnover of thyroid hormones. The turnover of thyroid hormones was studied after blocking the thyroidal iodide pump with potassium iodide. At 0 time, animals were given subcutaneous injections of 5μCi $[^{125}I]T_4$ (IM 141; Amersham, France; specific activity 1·2 mCi/mg)/kg body-weight or 10μCi $[^{125}I]T_3$ (IM 321; Amersham, France; specific activity 1·2 mCi/μg)/kg. At carefully timed intervals between 12 and 36 h after T_4 injection, and between 3 and 24 h after T_3 injection, blood samples were collected by venepuncture from the subclavian venous plexus (Higueret & Garcin, 1982).

The treatment of serum containing $[^{125}I]T_4$ (Geloso & Bernard, 1967) or serum containing $[^{125}I]T_3$ (Balsam *et al.* 1978) permitted the determination of the usual indices of peripheral metabolism of thyroid hormones (Ingenbleek & Malvaux, 1980): T_4 or T_3 distribution space (TDS), which is the virtual space in which the tracer dose of hormone would be found if it were distributed at a concentration equal to that of plasma; extrathyroidal T_4 or T_3 pool (ETP), which is the quantity of exchangeable T_4 or T_3 within the organism; T_4 or T_3 fractional turnover rate (K), which is the fraction of ETP replaced per unit time (this variable is calculated from the measured half-life ($t_{\frac{1}{2}}$) ($K = 0.693/t_{\frac{1}{2}}$); T_4 or T_3 disposal rate (TDR), which is the total hormone turnover.

Hormone assays. The assays were performed in duplicate on serum samples collected before initiation of turnover studies. Serum T_4 was determined by the competitive-binding assay of Murphy & Jachan (1965), adapted by Vigouroux (1972). Serum T_3 was determined by the specific double-antibody radioimmunoassay of Chopra *et al.* (1972) adapted by Jordan *et al.* (1980).

In vitro generation of T_3 from T_4 in liver homogenates

5'-Monodeiodinase activity was measured in liver homogenates by high-performance liquid chromatography (HPLC) of T_4 and T_3

Preparation of tissue fraction. Portions of liver were homogenized (1:2, w/v) in 0·1 M-Tris buffer (pH 7·4, 4°) and centrifuged at 9000 *g* for 4 min. The formation of T_3 was measured in the supernatant fractions by a modified method of Scammel *et al.* (1986).

Incubation and extraction conditions. Portions (100 μl) of the supernatant fraction were incubated for 30 min at 37° with T_4 (6·4 μM), $[^{125}I]T_4$ (0·5 μCi; specific activity of hormone 1·2 mCi/mg) and with or without reduced GSH (4·6 mM). The reaction was stopped by the addition of 2·3 vol. methanol which also served to extract the thyroid hormones. After centrifugation, the supernatant fraction was held at $-30°$ overnight and recentrifuged before analysis. Non-enzymic formation of T_3 was measured by incubation of the reaction mixture at 37° without liver homogenate: cold liver homogenate was added following incubation and thyroid hormones immediately extracted. Studies of the efficiency of extraction of $[^{125}I]T_3$ or $[^{125}I]T_4$ added to liver homogenates indicated that 97 (SE 2)% of radioactivity was extracted under these conditions.

Hormone separation. T_4 and T_3 were separated by an HPLC method modified from that of Hearn *et al.* (1978). The precise conditions were: column ultra-sphere ODS Beckman, 5 μm internal diameter, 250 mm length; mobil phase methanol:phosphoric acid (1 ml/l distilled–deionized water) (70:30, v/v); u.v. detection at 254 nm. The reaction products potentially quantified by this method are T_3, reverse T_3 (rT_3) and 3,5-diiodothyronine

Table 1. *Effects of a cysteine-deficient diet on different indices of peripheral metabolism of thyroxine (T_4)*

(Mean values with their standard errors)

Diet (g/kg)	No. of rats	TDS (ml/kg) Mean	SE	TT_4 (ng/ml) Mean	SE	ETP (ng/kg) Mean	SE	K Mean	SE	TDR (ng/kg per d) Mean	SE
Casein 120 + cysteine 1·93	12	182[a]	8	48[a]	3	9070[a]	400	0·99[a]	0·05	9090[a]	550
Casein 120	13	175[a]	9	43[a]	2	7560[b]	490	0·83[b]	0·05	6090[b]	430

TDS, T_4 distribution space; TT_4, total serum T_4; ETP, extrathyroidal T_4 pool; K, fractional turnover rate of T_4; TDR, T_4 disposal rate.
[a,b] Within columns, mean values with different superscript letters were significantly different (Student's *t* test): $P < 0.05$.

Table 2. *Effects of a cysteine-deficient diet on different indices of peripheral metabolism of 3,5,3'-triiodothyronine (T_3)*

(Mean values with their standard errors)

Diet (g/kg)	No. of rats	TDS (ml/kg) Mean	SE	TT_3 (ng/ml) Mean	SE	ETP (ng/kg) Mean	SE	K Mean	SE	TDR (ng/kg per d) Mean	SE
Casein 120 + cysteine 1·93	8	1910[a]	60	0·489[a]	0·013	930	30	1·99[a]	0·10	1850[a]	110
Casein 120	9	1670[b]	90	0·450[a]	0·007	750	50	1·93[a]	0·07	1450[b]	80

TDS, T_3 distribution space; TT_3, total serum T_3; ETP, extrathyroidal T_3 pool; K, fractional turnover rate of T_3; TDR, T_3 disposal rate.
[a,b] Within columns, mean values with different superscript letters were significantly different (Student's *t* test): $P < 0.05$.

Table 3. *Effects of a cysteine-deficient diet on hepatic 3,5,3'-triiodothyronine (T_3) generation*

(Mean values with their standard errors)

Diet (g/kg)	No. of rats	Liver GSH (μmol/g)		$[^{125}I]T_3$ formation (fmol/mg protein per min)			
				Basal		With 4·6 mM-GSH	
		Mean	SE	Mean	SE	Mean	SE
Casein 120 + cysteine 1·93	8	4·45[a]	0·24	44[a]	4	58[a]	6
Casein 120	8	2·17[b]	0·11	31[b]	1	45[a]	4

GSH, glutathione.

[a,b] Within columns, mean values with different superscript letters were significantly different (Student's t test): $P < 0.05$.

(T_2). Under the conditions of the present study, however, only negligible amounts of rT_3 and T_2 occurred and the results are expressed as fmol T_3 produced/mg protein per min.

Protein assay

Protein concentration of the homogenates was measured according to Bradford (1976) using the Bio-Rad protein assay (Bio-Rad Laboratories, West Germany).

Reduced GSH assay

Sample GSH was preserved with vinyl-2-pyridine (Griffith, 1980). GSH was measured enzymically according to Tietze's (1969) method, using glutathione reductase (*EC* 1.6.4.2) and 5,5'-dithiobis(2-nitrobenzoic) acid.

RESULTS

In vivo peripheral metabolism of T_4

The study of biological $t_{\frac{1}{2}}$ of T_4 in rats fed on a cysteine-deficient diet showed that $t_{\frac{1}{2}}$ was extended in relation to that of controls (20 (SE 1) v. 17·0 (SE 0·8) h). Table 1 summarizes the results of the T_4 turnover study. TDS, which is essentially the extracellular compartment, was not affected by the cysteine-deficient diet. However we observed a 16% decrease in ETP which is the quantity of exchangeable T_4 within the organism, and a great decrease (-33%) in TDR which corresponds to the rate of total T_4 turnover.

In vivo peripheral metabolism of T_3

The biological $t_{\frac{1}{2}}$ of T_3 was not modified by the cysteine-deficient diet (8·7 (SE 0·37) v. 8·5 (SE 0·45) h for control diet). The values for T_3 turnover indices are presented in Table 2. TDS for T_3 was greater than that of T_4 because its cellular internalization rate and consequently its cellular concentration is greater (De Groot, 1979). In the animals receiving the cysteine-deficient diet, the TDS was significantly decreased (-13%) in comparison with that of controls. ETP for T_3 was decreased (-19%) in rats fed on a cysteine-deficient diet. The findings suggest a change in peripheral metabolism of T_4 which constitutes the major source of T_3 in man and experimental animals (Chopra *et al.* 1982). For T_4, we observed a decrease (-22%) in TDR in rats which received the cysteine-deficient diet compared with control rats.

In vitro generation of T_3 from T_4 and the influence of GSH

As can be seen in Table 3, the cysteine-deficient diet caused a great decrease (-51%) in hepatic GSH. Under basal conditions, i.e. in the absence of GSH in the reaction mixture, the $[^{125}I]T_3$ formation by the liver was less (-30%) in rats fed on the cysteine-deficient diet. After addition of GSH to the incubation medium, T_3 generation was increased in the two groups of rats (32% increase in controls, 45% increase in cysteine-deficient animals). However, liver T_3 generation was not significantly different between the two groups of animals when GSH was added to the medium as a cofactor.

DISCUSSION

The decrease in T_4 ETP observed in rats which received the cysteine-deficient diet would suggest an alteration of thyroid hormone synthesis. The molecular structure of thyroglobulin includes many half-cystine residues forming intrachain or interchain disulphide bonds (De Crombrugghe *et al.* 1966), more particularly at the carboxyl-end of the protein (Di Lauro *et al.* 1984). Recently it was demonstrated that hormogenic sites are located at the extremities of thyroglobulin (Lejeune *et al.* 1983; Rawitch *et al.* 1984). Possibly cysteine depletion caused by the diet led to a change in thyroglobulin which affected T_4 biosynthesis.

The decreased TDR in the rats fed on the cysteine-deficient diet is consistent with an alteration of peripheral metabolism of T_4. T_4 is metabolized primarily by deiodination, mainly in the liver and kidney (for review, see Köhrle *et al.* 1987). The extrathyroidal 5'-monodeiodination of T_4 to T_3 is enzymic in nature (Visser *et al.* 1976) and relies on GSH as a cofactor (Imai *et al.* 1980). The decrease in T_4 deiodination as well as the decrease in hepatic GSH observed in our experiments tend to confirm this explanation. The decrease in hepatic GSH found in rats fed on the unsupplemented diet is presumed to be the result of cysteine insufficiency for the biosynthesis of this tripeptide (γ-L-glutamyl-L-cysteinyl-glycine). Moreover, GSH is well-known as a cysteine reservoir during cystine depletion in growing rats (Cho *et al.* 1984).

5'-Monodeiodination of T_4 to T_3 by peripheral tissues accounts for most of the daily production of T_3 in the rat (Abrams & Larsen, 1973). Studies using homogenates have shown that on a per gram basis liver and kidney are much more potent in monodeiodination of T_4 to T_3 than other tissues (Chopra *et al.* 1978). In whole-body terms, liver is the most important source of T_3 in peripheral tissues. The decrease in this metabolic pathway in the rats fed on the cysteine-deficient diet explains the diminution of T_3 ETP observed in these animals. T_3 is converted to $3,3'\text{-}T_2$ and $3,5\text{-}T_2$ (diiodothyronine) by an enzymic monodeiodination (Chopra *et al.* 1978) and a slowing down of these reactions may be responsible for the decrease in TDR found in the animals fed on the unsupplemented diet.

With regard to TDS, the T_3 distribution in the body is essentially intracellular. The T_3 uptake by target cells is the result of active mechanisms (Rao *et al.* 1976; Cheng, 1983). However, we have shown that GSH was involved in cellular T_3 uptake. A decrease in intracellular GSH leads to a reduction in the T_3 penetration of cells (Higueret & Garcin, 1984). In the present experiment the hepatic GSH is decreased and therefore a decrease in T_3 TDS would not be unexpected. The cysteine-deficient diet caused a tissue deficiency of sulphydryl groups, the consequences of which may be translated into various effects on thyroid hormone metabolism. It is now necessary to determine if the hormonal metabolic changes observed have effects on the amount of free T_3, which is the biologically active form of the thyroid hormones.

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