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Effect of selenium deficiency on hepatic type I 5-iodothyronine deiodinase activity and hepatic thyroid hormone levels in the rat

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Selenium deficiency in rats for a period of up to 6 weeks inhibited both the production of 3,3′,5-tri-iodothyronine (T_3) from thyroxine (T_4) (5′-deiodination) and also the catabolism of T_3 to 3,3′-di-iodothyronine (5-deiodination) in liver homogenates. The hepatic stores of T_3 were decreased by only 8% in selenium deficiency, despite the T_3 production rate from T_4 being only 7% of the rate found in selenium-supplemented rats. Hepatic glutathione *S*-transferase (GST) activity was increased in both hypothyroidism and selenium deficiency, but apparently by different mechanisms, since mRNA expression for this family of enzymes was lowered by hypothyroidism and increased in selenium deficiency. It is concluded that, since both T_3 production and catabolism are inhibited by selenium deficiency, there is little change in hepatic T_3 stores, and therefore the changes in the activity of certain hepatic enzymes, such as GST, that are found in selenium deficiency are not the result of tissue hypothyroidism.

INTRODUCTION

All thyroxine (T_4) is synthesized in the thyroid gland, but T_4 is regarded as a prohormone requiring 5′-monodeiodination to produce the active hormone, 3,3′,5-tri-iodothyronine (T_3). T_4 may also undergo 5-monodeiodination to produce the metabolically inactive isomer reverse T_3 (rT_3). T_3 and rT_3 are metabolized further by 5- and 5′-deiodination respectively to produce 3,3′-di-iodothyronine (T_2) [1].

More than 80% of plasma T_3 is produced by 5′-deiodination of T_4 in non-thyroidal tissues, particularly the liver, kidney and muscle; in these tissues the reaction is catalysed by type I iodothyronine deiodinase (ID-I). ID-I has not been purified, but there is much indirect evidence that the enzyme can catalyse both 5- and 5′-monodeiodination of iodothyronines [1,2]. We have demonstrated that hepatic ID-I is a selenoenzyme [3–6], and it has also been shown that ID-I contains stoichiometric amounts of selenium (Se) [7]. Cloning of the enzyme has confirmed the presence of a selenocysteine residue at the active site [8].

Brain, pituitary gland and brown adipose tissue contain separate enzyme systems for iodothyronine deiodination, with 5′- and 5-deiodination being catalysed by type II and type III deiodinases respectively, both enzymes being distinct from ID-I [1,2]. ID-II is not a selenoenzyme, although its activity is diminished in Se-deficient euthyroid rats [4,9].

In Se-deficient rats, hepatic and renal 5′-ID-I activty is approx. 10-fold lower than the activity found in Se-supplemented animals, yet plasma T_3 falls by only approx. 10% [3–5]. This disparity could be explained if 5-ID-I activity and production of T_2 was also inhibited by Se deficiency, but this possibility has not been investigated.

Hepatic levels of the thyroid hormones T_3 and T_4 have not been measured in Se deficiency, although the absence of decreases in malic enzyme and α-glycerophosphate dehydrogenase, enzymes that are modified by thyroid status, suggests that Se deficiency may not produce tissue hypothyroidism; the activities of these enzymes are decreased by propylthiouracil(PTU)-induced hypothyroidism but are increased in Se deficiency. In contrast, the activity of hepatic glutathione *S*-transferase (GST) is increased by both Se deficiency and hypothyroidism [10].

The GSTs are a group of dimeric enzymes with several biochemical functions, the most important of which is probably detoxification. The major GST subunits in the rat are Ya, Yb_1, Yb_2 and Yc; the Ya and Yc subunits belong to the Alpha class and the Yb_1 and Yb_2 subunits belong to the Mu class [11,12]. Using specific radioimmunoassay we have shown that Se deficiency increases the hepatic content of each of these subunits [13]. Rat liver also contains small amounts of the Yf subunit of the Pi class, but this subunit has been shown by immunohistochemistry to be present in the biliary epithelia and not the hepatocyte.

In the present study we have addressed three questions to obtain a clearer understanding of the factors involved in the altered hepatic enzyme expression observed in Se deficiency: (i) is hepatic 5-deiodination of T_3 impaired in Se deficiency?, (ii) what are the hepatic and plasma levels of T_3 and T_4 in Se deficiency? and (iii) what are the effects of Se deficiency and hypothyroidism on the hepatic RNA for the Mu and Alpha class GST in the rat?

MATERIALS AND METHODS

Materials

Antisera used for the measurement of plasma thyroid hormone concentrations were from the Scottish Antibody Production Unit (Carluke, Lanarkshire, Scotland, U.K.). ^{125}I-labelled T_3 and T_4 (specific radioactivity > 1200 μCi/μg), hybridization transfer membranes (nylon, 0.45 μm) and [α-^{32}P]dCTP (specific radioactivity 3000 Ci/mmol) were from Amersham International (Aylesbury, Bucks., U.K.), DNA polymerase 1 (Klenow fragment) was from Bethesda Research Laboratories. All other reagents were from Sigma or BDH (both of Poole, Dorset, U.K.).

Animals and diets

Weanling hooded Lister rats of the Rowett strain were used.

Abbreviations used: T_4, thyroxine; T_3, 3,3′,5-tri-iodothyronine; rT_3, reverse T_3; T_2, 3,3′-di-iodothyronine; ID, iodothyronine deiodinase; GST, glutathione *S*-transferase; PTU, propylthiouracil; DTT, dithiothreitol; CDNB, 1-chloro-2,4-dinitrobenzene; GSH-Px, glutathione peroxidase.

For the investigation of 5- and 5′-ID-I activity, two groups of six animals were fed either a synthetic diet containing $< 5\ \mu g$ of Se/kg or the same diet supplemented with $100\ \mu g$ of Se/kg as Na_2SeO_3 for 6 weeks [3].

To investigate the effects of Se deficiency and hypothyroidism on hepatic GST mRNA, four groups of six animals were used. The control and Se-deficient groups were fed the diets described above, and in addition two further groups (Se-deficient and Se-sufficient groups) were rendered hypothyroid by giving 0.5% PTU in the drinking water for the 5 weeks of the study [10].

All animals were housed in plastic cages with stainless steel grid tops and floors, with distilled water and food provided *ad libitum*.

Preparation of plasma and liver homogenates

Under ether anaesthesia, blood was collected into heparinized tubes and the plasma separated by centrifugation at 1500 *g* for 15 min at 4 °C. Livers were perfused via the portal vein with KCl (150 mmol/l) and frozen immediately in liquid nitrogen. Liver and plasma were then stored at −85 °C until required.

To prepare cytosol for the measurement of GST activity, portions of liver were homogenized in potassium phosphate buffer, pH 7.4 (125 mmol/l), pH 7.5, using a Teflon pestle/glass body homogenizer, and the cytosol was obtained by centrifugation at 105000 *g* for 60 min.

Liver homogenates used for determining ID-I activity were prepared in potassium phosphate buffer (125 mmol/l), pH 7.4, containing EDTA (1 mmol/l) and dithiothreitol (DTT) (20 mmol/l).

The Se-deficient diet used produces marked Se deficiency in rats fed for periods in excess of 4 weeks, as indicated by hepatic glutathione peroxidase activity being less than 1% of the activity found in rats fed the Se-supplemented diet [3–6,10].

Measurement of hepatic and plasma T_3 and T_4

Weighed portions of liver were homogenized in 4 vol. of NH_4OH (20 mmol/l)/methanol (1:400, v/v) using a Teflon pestle/glass body homogenizer. The homogenates were left to extract overnight at 4 °C and then centrifuged at 3000 *g* for 15 min. The concentrations of T_3 and T_4 in the methanol extracts and in unextracted plasma were determined by radioimmunoassay [14].

Hepatic enzyme and protein measurements

Cytosolic GST activity was determined on a centrifugal analyser with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate [15]. Cytosolic glutathione peroxidase activity was measured with H_2O_2 (0.25 mmol/l) as substrate in the presence of GSH (5 mmol/l) [16]. 5′-ID-I activity was measured in liver homogenates by radioimmunoassay of the T_3 produced from added T_4 [3,4].

The 5-ID-I activity in liver was measured by determining the proportion of T_3 converted into T_2. Portions of homogenate (0.3 ml) were incubated at 37 °C with DTT (20 mmol/l) and 20 μCi of [^{125}I]T_3. Immediately after the addition of substrate (time zero) and at 3 h and 20 h, methanol (0.5 ml) was added and the precipitate was removed by centrifugation at 3000 *g* for 30 min. The methanolic supernatants were evaporated to dryness under nitrogen and the residue was redissolved in 50 μl of methanol. The methanolic extracts were transferred to a 20 cm cellulose plastic-backed t.l.c. plate and the chromatograms were developed in a solvent system of t-butanol/2 M-NH_4OH/chloroform (188:35:30, by vol.). The plates were then scanned on a Berthold Thin Layer Chromatogram Autoradiograph Scanner and the two peaks corresponding to T_3 (R_F 0.33) and T_2 (R_F 0.18) were identified and counted for radioactivity in an LKB Multigamma counter (Pharmacia, Milton Keynes, U.K.). The percentage of radioactive T_3 converted to T_2 over the 20 h incubation period was calculated. Control incubations were also performed with boiled homogenates.

Northern blotting

Approximately equal portions of liver from six rats in each group were pooled and the total RNA was isolated from the livers using the guanidine hydrochloride method as described by Cox [17]. Both the concentration and the purity of the RNA were estimated spectrophotometrically, and the RNA was visualized for equal loading and integrity by staining a denaturing formaldehyde gel with ethidium bromide and exposure to u.v. light. Sample loadings were thereafter adjusted as appropriate and a further assessment was made by probing with a mouse actin cDNA, as the expression of actin mRNA appears to be unaffected by Se or thyroid status [18,19].

RNA was separated on denaturing formaldehyde gels prior to determining the content of Alpha, Mu and Pi class GST mRNA using hybridization conditions described previously [20]. The cDNA probes were labelled by random priming [21,22]. The full-length human Alpha class GST was as described by Lewis *et al.* [23] and was identical to that isolated by Board & Webb [24]. The full-length human Pi GST [25] was a gift from Professor M. Muramatsu (Biochemistry Department, University of Tokyo). The 1000 bp Mu class GST fragment was kindly donated by Dr. J. Taylor (CRC Molecular Toxicology, Middlesex Hospital, London, U.K.) and contains exons 3, 4 and 5 of the human Mu-class gene. mRNA obtained from a human sarcoma cell line (DX5) which expresses both Mu and Pi class GST was used as a control for the blots.

A mouse actin processed pseudogene [26] was used to assess loadings. All blots were washed at 65 °C with 2 × SSC (0.3 M-NaCl, 0.03 M-trisodium citrate, pH 7.4) and 0.1% SDS. Bands were visualized by exposure to X-ray film and quantified by scanning and integration on a Chromoscan 3 densitometer. The ratio of integrals of the actin and GST blots was calculated.

RESULTS

Effects of Se deficiency on hepatic 5- and 5′-ID-I activities and hepatic and plasma T_3 and T_4

The production of T_3 from added T_4 (5′-ID-I activity) was markedly inhibited by Se deficiency, with the activity in Se-deficient animals being only approx. 7% of the activity found in Se-supplemented animals (Table 1).

Table 1. Effect of Se deficiency on plasma and hepatic T_4 and T_3 levels and hepatic 5- and 5′-ID-I activities

Rats were fed diets for 6 weeks from weaning. Results are means ± S.D. ($n = 5$). Significant differences between Se-deficient and Se-supplemented rats are given: * $P < 0.05$, ** $P < 0.001$.

	+Se	−Se
Plasma		
T_4 (nmol/l)	72.8 ± 9.0	116.6 ± 9.0**
T_3 (nmol/l)	1.82 ± 0.30	1.54 ± 0.19*
Liver		
T_4 (ng/g)	14.82 ± 1.32	25.65 ± 3.21*
T_3 (ng/g)	1.80 ± 0.06	1.40 ± 0.04**
5′-ID-I (fmol of T_3/min per mg of protein)	112.9 ± 7.6	7.7 ± 0.79**
5-ID-I (% of T_3 converted in 18 h)	20.2 ± 7.6	5.6 ± 2.3*

Table 2. Thyroid hormone, enzyme and RNA measurements in Se deficiency and PTU-induced hypothyroidism

Rats (six per group) were fed Se-deficient or Se-supplemented diets *ad libitum* for 5 weeks. Hypothyroid animals received PTU for the 5 weeks of the study. Means ± S.D. are shown and the ratio of GST RNA to actin RNA is shown for the RNA data. Differences between the Se-supplemented euthyroid group and other groups are given by $*P < 0.05$ and $**P < 0.001$.

	Control		PTU	
	+Se	−Se	+Se	−Se
Plasma				
T_4 (nmol/l)	88.5±9.6	116.7±8.6**	4.5±0.7**	10.2±2.8*
T_3 (nmol/l)	1.3±0.28	1.0±0.34*	< 0.1**	< 0.1**
Liver enzymes				
GSH-Px (units/mg of protein)	1.08±0.16	0.005±0.001**	1.4±0.2**	0.02±0.006**
GST (μmol of CDNB/mg of protein)	0.33±0.09	0.54±0.11**	0.69±0.19**	0.75±0.27**
Liver GST RNA/actin RNA				
Alpha class GST	1.64	2.34	0.78	1.60
Mu class GST	1.55	1.78	0.81	1.48

The conversion of T_3 to T_2 (5-ID-I activity) in liver homogenates was also inhibited by Se deficiency. After incubation for 3 h, no significant metabolism of T_3 was detected in Se-deficient animals. After 20 h, 20% of the T_3 had been converted to T_2 in liver homogenates from the Se-supplemented animals, whereas only 5.6% had been converted in the homogenates from Se-deficient rats.

Se deficiency produced small but significant decreases in hepatic and plasma T_3 levels and significant increases in both plasma and hepatic T_4 levels.

Effects of Se deficiency and hypothyroidism on plasma thyroid hormone concentrations and hepatic enzyme and GST mRNA levels

Plasma thyroid hormones and liver enzymes. In rats fed the Se-deficient diet, hepatic cytosolic glutathione peroxidase (GSH-Px) activity was decreased to less than 1% of the activity found in rats fed the Se-supplemented food, confirming that Se deficiency had been produced in the former group of animals (Table 2). The induction of hypothyroidism with PTU significantly increased GSH-Px activity in both Se-deficient and Se-sufficient animals when compared with euthyroid Se-deficient and Se-sufficient rats. The activity of GST was increased significantly by both Se deficiency ($P < 0.001$) and PTU treatment ($P < 0.05$). The effect of PTU was evident in both Se-deficient and Se-replete animals ($P < 0.01$), the effect being additive.

Se deficiency produced significant increases and decreases respectively in plasma total T_4 and total T_3 in rats not receiving PTU. In rats receiving PTU, T_3 was undetectable and total T_4 was considerably decreased, confirming the hypothyroid state of the animals.

Northern blotting

Se deficiency in euthyroid rats increased the amounts of Mu class and Alpha class GST mRNA by 15% and 43% respectively, as determined by densitometry of the autoradiographs (Table 2 and Fig. 1). Induction of hypothyroidism with PTU in Se-sufficient rats decreased the level of both Mu and Alpha class GST mRNA by about 50%. Hypothyroidism also decreased GST mRNA in Se-deficient animals, the concentrations of the Mu and Alpha class GST mRNAs being 83% and 68% of those in Se-deficient euthyroid animals. In hypothyroid rats, Se deficiency increased the levels of Mu and Alpha class GST RNAs by 83% and 205% respectively.

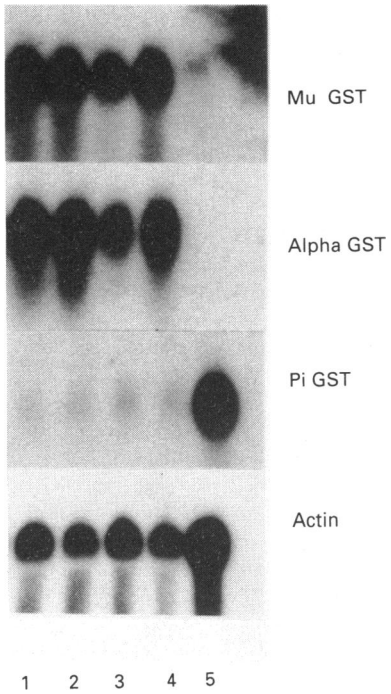

Fig. 1. Hepatic GST mRNA levels in Se-deficient, Se-supplemented and hypothyroid rats

Northern blot analysis was carried out as described in the Materials and methods section. Lane 1, +Se; lane 2, −Se; lane 3 +Se+PTU; lane 4, −Se+PTU. DX5 mRNA (lane 5) is from a human sarcoma cell line which expresses both Mu and Pi class GST and was used as a control.

The expression of Pi class GST and mRNA was too low for quantification in all groups of rats.

DISCUSSION

Using affinity labelling, we have shown that the loss of hepatic 5′-ID-I activity in Se deficiency results not from inhibition but from decreased synthesis of the enzyme [6]. It is now apparent that Se deficiency results in an inhibition of both 5- and 5′-ID-I activities in the liver (Table 1), supporting the concept that both

of these activities are incorporated into a single enzyme [1,2]. This explains, at least in part, why plasma T_3 fell by only approx. 10% despite hepatic synthesis of T_3 decreasing by over 90% in Se-deficient rats.

The rate of T_3 production *in vitro* measured in rat liver homogenates (Table 1) was far greater than the rate of T_3 degradation. This marked difference in rates may be explained by the assay methods used. The production of T_3 was determined in the presence of 5 μmol of T_4/litre, which is approximately twice the K_m for the enzyme (K_m 2.3 μmol/l), whereas the degradation of T_3 was determined at a substrate concentration of approx. 0.1 μmol/l, which is far less than the K_m of ID-I for this substrate (K_m 6.2 μmol/l). It was not possible to use higher concentrations of T_3 as substrate, since this resulted in a very low specific activity of the T_3 tracer. This would have made it difficult to determine accurately the rate of T_3 degradation unless long incubation periods were used.

The thyroid status of the liver is governed by the tissue concentration and not the plasma concentration of T_3. The present results show that, as in plasma, Se deficiency causes a small but significant decrease in hepatic T_3 concentration. Similarly, the rise in plasma T_4 found in Se deficiency was mirrored by a similar rise in hepatic T_4. In this study we have measured the total plasma concentrations of thyroid hormones; however, we have shown previously that the changes in the unbound fraction of T_4 in plasma (free T_4) mirror the changes seen in total T_4 in Se deficiency, suggesting that the concentration of plasma T_4-binding protein is largely unaffected by Se status [27].

Se deficiency and hypothyroidism both increase the activities of the Alpha and Mu GST classes, suggesting that the changes are linked by a common mechanism, especially as plasma T_3 is decreased in both situations [10]; the mRNA data, however, contradict this hypothesis. In euthyroid rats Se deficiency increased the expression of the hepatic mRNA coding for both the Alpha and Mu class GSTs (Table 2); these results are in accordance with the increase in hepatic GST YaYa, GST Yb1Yb1, and GST YcYc measured by radioimmunoassay in Se-deficient rats [13]. Hypothyroidism, however, had the opposite effect to Se deficiency as it decreased the mRNA of both Alpha and Mu class GSTs. The mRNA for both classes of GST was induced in euthyroid and hypothyroid rats by Se deficiency, further suggesting that the mechanisms controlling GST mRNA production in Se deficiency and hypothyroidism are independent. These results support the view that the changes in hepatic GST expression in Se deficiency do not occur as a result of tissue hypothyroidism.

Although the activity of GST increases in rats or mice made hypothyroid by either PTU treatment or surgical thyroidectomy [10,28,29], the mRNAs for the Alpha and Mu class enzymes were considerably decreased by hypothyroidism. These results suggest that the expression of the GST is under the control of thyroid hormones and that the increased concentration of hepatic GST found in hypothyroidism results from stabilization, probably by decreased degradation, of the protein in the hypothyroid state and not from the increased transcription. Using a pulse-labelling technique with [^{14}C]guanidine/arginine followed by immunoprecipitation, Arias *et al.* [28] demonstrated that the increase in the hepatic Alpha class GST in thyroidectomized animals was associated with an increase in its half-life from 2.3 to 5.1 days; these changes could be reversed by administration of thyroid hormones.

In conclusion, the present data indicate that although Se deficiency can alter the expression of hepatic GST mRNA and its enzyme activity, this is not caused by significant tissue hypothyroidism.

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