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Inhibition of hepatic deiodination of thyroxine is caused by selenium deficiency in rats

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Selenium (Se) deficiency produced up to a 14-fold decrease in hepatic tri-iodothyronine (T_3) production from thyroxine (T_4) *in vitro*. The T_3 production rate could not be restored by the addition of a variety of co-factors, nor by the addition of control homogenate. The impairment in hepatic T_3 production observed in Se deficiency was reflected in the concentrations of thyroid hormones circulating in plasma, T_4 being increased approx. 40% and T_3 being decreased by 30%. However, the fall in plasma T_3 concentrations was smaller than might be expected in view of the marked decrease in T_3 production. Se deficiency had no measurable effect on plasma reverse-tri-iodothyronine concentrations. The data suggest that Se deficiency produces an inhibition of both 5- and 5'-deiodination, consistent with the widely held view that these reactions are catalysed by the same enzyme complex. The mechanism of inhibition appears not be mediated by changes in thiol levels, but a direct role of Se in the activity of the deiodinase complex cannot be excluded.

INTRODUCTION

Selenium (Se) deficiency has important pathological sequelae in both animals and in man. Animals deficient in the trace nutrient may exhibit poor growth, impaired neutrophil function and increased susceptibility to infection [1–3]. In the presence of concurrent vitamin E deficiency, animals may develop liver necrosis or myopathy [4,5]. In man, lowered Se status has been associated with cardiomyopathy and an increased risk of developing certain cancers [6,7].

In animals and man only one functional selenoprotein, selenium-dependent glutathione peroxidase (Se-GSH-Px), has been characterized, and is generally thought to be involved in protecting the cell membrane from peroxidative damage [4,5,8]. In the early stages of Se deficiency the activity of Se-GSH-Px is markedly decreased, but with prolonged deficiency the activity of several hepatic enzyme systems are altered, some being decreased (e.g. *N*-demethylase, NADPH: cytochrome *c* reductase) and others increased [glutathione *S*-transferase (GST), ethoxycoumarin de-ethylase] [9–11].

The many biochemical changes which occur in Se deficiency cannot be fully explained by loss of Se-GSH-Px activity. For instance, elevated GST activity in Se-deficient animals can be restored to control values by small doses of Se which do not affect cytosolic Se-GSH-Px activity [9]. However, thyroid status affects the expression of many enzymes, and hypothyroidism can affect GST activity in a similar fashion to Se deficiency [12].

Thyroxine (T_4) is synthesized only in the thyroid, but 85% of the circulating 3:5,3'-tri-iodothyronine (T_3) is produced in peripheral tissue (mainly the liver) by 5'-(outer-ring)monodeiodination of T_4. T_4 may also undergo 5-(inner-ring)deiodination to produce the metabolically inactive isomer 3,3':5'-tri-iodothyronine, also known as 'reverse T_3' (rT_3); about 90% of circulating rT_3 is derived from this reaction rather than from synthesis *de novo* in the thyroid. Peripheral deiodination is, therefore, an important determinant of metabolic status, since, without it, T_3 cannot be produced in any quantity (for review see [13,14]).

Two types of enzyme responsible for 5'-monodeiodination of thyroxine in non-thyroidal tissue have been described. The type I enzyme occurs mainly in the liver and kidney and is inhibited by low concentrations of propylthiouracil. The type II enzyme is found mainly in the pituitary, brown adipose tissue and brain, and has different inhibition characteristics with propylthiouracil. Both the type I and type II deiodinase enzymes are activated by dithiothreitol (DTT) [15].

In the present paper we report the effects of Se deficiency on plasma thyroid-hormone concentrations and on the hepatic production of T_3 from T_4 *in vitro*.

MATERIALS AND METHODS

Reagents

Antisera for T_3 and T_4 measurement were obtained from the Scottish Antibody Production Unit (Carluke, Lanarkshire, Scotland, U.K.), and ^{125}I-labelled T_3 and T_4 (sp. radioactivity > 1200 μCi/μg) were from Amersham International (Amersham, Bucks., U.K.). The radioimmunoassay kit for measuring rT_3 was from Immunodiagnostics Ltd. (Washington, Tyne and Wear, U.K.). All other reagents were from Sigma Chemical Co. or BDH (both of Poole, Dorset, U.K.).

Animals and diets

Weanling male Hooded Lister rats of the Rowett strain, 21 days old, were used in all experiments except one, in which Wistar rats (Bantin and Kingman, Hull,

Abbreviations used: T_3, 3:5,3'-tri-iodothyronine; T_4, thyroxine; rT_3, reverse T_3 (3,3':5'-tri-iodothyronine); Se-GSH-Px, Se-dependent glutathione peroxidase; GST, glutathione *S*-transferase; DTT, dithiothreitol.

U.K.) were used. The animals were group-housed in plastic cages with stainless-steel grid tops and floors, and food and distilled water were available *ad libitum*. Animals were fed a synthetic diet of the following composition: sucrose, 71.8 g; amino acid mixture, 18.0 g; lard, 3.5 g; cod liver oil, 1.5 g; and a vitamin/mineral/trace-metal mixture, 5.2 g. The amino acid mixture contained the following: L-alanine, 5.0 g; L-arginine hydrochloride, 6.0 g; L-asparagine, 4.0 g; L-aspartic acid, 5.0 g; L-cystine, 2.0 g; glycine, 5.0 g; L-histidine, 3.0 g; L-isoleucine, 5.0 g; L-leucine, 7.5 g; L-lysine hydrochloride, 7.0 g; L-methionine, 4.0 g; L-monosodium glutamate, 30.0 g; L-phenylalanine, 5.0 g; L-proline, 4.0 g; L-serine, 5.0 g; L-threonine, 5.0 g; L-tryptophan, 1.5 g; L-tyrosine, 3.0 g; and L-valine, 6.0 g/113.0 g mix. The vitamins, minerals and trace elements used were as described by Abdel-Rahim *et al.* [16], and Na_2SeO_3 was omitted where appropriate.

Selenium-deficient groups (−Se) received the basal diet containing less than 0.005 mg of Se/kg, and the control groups (+Se) received the same diet supplemented with 0.1 mg of Se/kg as Na_2SeO_3. All diets contained tocopherol acetate (200 mg/kg) and copper as $CuSO_4,5H_2O$ (10 mg of copper/kg).

Preparation of plasma and liver homogenates

At 4, 5 or 6 weeks after weaning, rats were anaesthetized with diethyl ether, and blood was collected by cardiac puncture into heparinized tubes. After centrifuging the blood at 1500 *g* for 15 min, the plasma was stored at −85 °C for subsequent measurement of total T_3, T_4 and rT_3 concentrations.

Liver homogenates were prepared by the method of Visser *et al.* [17] by homogenizing individual livers in 3 vol. of sucrose/Tris buffer (0.25 M-sucrose in 0.05 M-Tris/HCl, pH 7.4) using a Teflon pestle/glass-body homogenizer. In experiments with Wistar rats, fresh livers were used for homogenate preparation. With Hooded Lister rats, livers were perfused via the portal vein with 0.15 M-KCl, frozen in liquid N_2 and stored at −85 °C. The frozen livers were thawed, when required for homogenization, by placing them in 3 vol. of sucrose/Tris buffer.

Complete homogenates were used for the measurement of T_3 production, but a portion of the homogenates was centrifuged at 100000 *g* for 60 min and the supernatants were used for the measurement of GST and Se-GSH-Px activity. Protein was measured in homogenates and the 100000 *g* supernatants by the biuret reaction.

Measurement of hepatic T_3 production

The production of T_3 from added T_4 in liver homogenates was measured by a radioimmunoassay employing a system based on the method of Visser *et al.* [17]. Homogenates (2 ml) were placed into glass tubes and the tubes incubated at 37 °C for 5 min before the addition of 10 μg of T_4 dissolved in 20 μl of phosphate buffer (0.5 M; pH 7.4). At increasing time periods, 0.2 ml portions of the homogenates were taken into 0.4 ml of ethanol and mixed for 30 s on a vortex mixer. The ethanolic extracts were then centrifuged at 1500 *g* for 15 min, and the T_3 concentration of the supernatants was measured by radioimmunoassay after 10-fold dilution with sucrose/Tris buffer.

Control incubations containing either sucrose/Tris buffer or homogenate heated to 60 °C for 30 min were done for each experiment. These controls allowed correction for cross-reactivity of the added T_4 in the T_3 radioimmunoassay.

Radioimmunoassay of T_3, T_4 and rT_3

The concentrations of total T_3 and total T_4 in plasma and the concentrations of T_3 in ethanolic extracts of incubations were measured by using double-antibody radioimmunoassays [18]. Both assays had an intra-assay coefficient of variation of $< 10\%$ over the ranges of T_4 and T_3 concentrations determined.

The concentration of rT_3 in plasma was measured by using a kit method which had a minimum detection limit of 15 pmol/litre and an intra-assay coefficient of variation of $< 10\%$.

GST and Se-GSH-Px measurements

The activity of GST in 100000 *g* supernatants was measured with 1-chloro-2,4-dinitrobenzene and GSH as substrate at 25 °C [19]. Se-GSH-Px activity was measured, with 0.25 mM-H_2O_2 as substrate, in the presence of 5 mM-GSH [20].

Statistical analysis

Results were compared by using the non-parametric Mann–Whitney U-test.

RESULTS

Rat weights

In experiments with Hooded Lister rats fed for 4, 5 and 6 weeks, the weights of Se-sufficient (+Se) and -deficient (−Se) animals were not significantly different (Table 1). Wistar rats were not weighed.

GST and Se-GSH-Px activity

When compared with control animals, the consumption of an Se-deficient diet for 4 or 6 weeks produced an approximately 2-fold increase in hepatic GST activity and a 98% decrease in hepatic Se-GSH-Px activity (Table 1), confirming Se deficiency in the rats.

Plasma T_3, T_4 and rT_3 concentrations

Consumption of an Se-deficient diet by rats for 4, 5 or 6 weeks produced a significant increase in plasma T_4 concentration and a significant decrease in plasma T_3, but no significant change in plasma rT_3 was observed at 5 weeks. rT_3 was not measured at other times (Table 2).

After 6 weeks on the Se-deficient diet, Wistar rats showed a mean increase in T_4 of 38% and a mean fall in T_3 of 32%. In Hooded Lister rats, only 4 weeks of Se deficiency increased plasma T_4 by 57% and decreased plasma T_3 by 15%.

Hepatic production of T_3

***(a)* No added cofactors.** After the addition of T_4 to incubations of liver homogenates, T_3 production remained linear for approx. 10 min in both Se-deficient and control animals. For all subsequent experiments the rate of T_3 production was therefore calculated from triplicate determinations of the T_3 produced at 10 min corrected for the endogenous T_3 measured in triplicate determinations at zero time.

The data from the three groups of animals are shown

Table 1. Body weights and GST and Se-GSH-Px activities in 100000 g supernatant from control (+Se) and Se-deficient (−Se) animals

Body weights are means ± S.D. for the numbers of rats given in parentheses. Wistar rats were not weighed (NW). GST activities are expressed as μmol of chlorodinitrobenzene–GSH conjugate formed/min per mg of cytosolic protein. Se-GSH-Px activities are expressed as μmol of NADPH oxidized/min per mg of protein. Results are means ± S.D. for the numbers of animals given in parentheses. Abbreviation used: ND, not determined. Significance of differences from control animals: $^{**}P < 0.005$; $^{***}P < 0.001$.

Strain	Duration of dietary treatment (weeks)	Body weight (g)		GST		Se-GSH-Px	
		+Se	−Se	+Se	−Se	+Se	−Se
Hooded Lister	4	175.0 ± 15.8 (8)	169.0 ± 12.1 (10)	0.35 ± 0.06 (8)	0.52 ± 0.15 (10)**	1.158 ± 0.200 (8)	0.008 ± 0.006 (10)***
	5	226.0 ± 19.6 (6)	233.0 ± 16.5 (6)	ND	ND	ND	ND
	6	241.0 ± 19.1 (7)	250.0 ± 24.9 (7)	0.28 ± 0.03 (7)	0.62 ± 0.08 (7)***	1.365 ± 0.180 (7)	0.006 ± 0.005 (7)***
Wistar	6	NW	NW	0.40 ± 0.04 (6)	0.96 ± 0.18 (6)***	1.549 ± 0.110 (6)	0.010 ± 0.015 (6)***

Table 2. Plasma T_4, T_3 and rT_3 concentrations in control (+Se) and Se-deficient (−Se) rats

Results are means ± S.D. for the numbers of animals given in parentheses; –, not measured. Significance of the difference from control animals: $^{*}P < 0.05$; $^{**}P < 0.005$, $^{***}P < 0.001$; ^{NS}, not significant.

Strain	Duration of dietary treatment (weeks)	Plasma thyroid-hormone concn.					
		+Se			−Se		
		T_4 (nmol/l)	T_3 (nmol/l)	rT_3 (pmol/l)	T_4 (nmol/l)	T_3 (nmol/l)	rT_3 (pmol/l)
Hooded Lister	4	73.5 ± 9.7 (8)	1.3 ± 0.07 (8)	–	115.8 ± 15.2 (10)***	1.1 ± 0.15 (10)**	–
	5	81.8 ± 6.8 (6)	1.5 ± 0.14 (6)	121 ± 42 (6)	118 ± 6.8 (6)***	1.2 ± 0.29 (6)*	106 ± 32 (6)^{NS}
	6	108.5 ± 21.0 (7)	1.8 ± 0.18 (7)	–	147.6 ± 20.4 (8)*	1.5 ± 0.16 (7)**	–
Wistar	6	63.0 ± 9.1 (6)	1.3 ± 0.17 (6)	–	87.2 ± 9.2 (6)***	0.9 ± 0.11 (6)**	–

Table 3. Effect of Se deficiency on hepatic T_3 production

Animals were fed on an Se-deficient (−Se) or a control (+Se) diet for either 4 or 6 weeks before the measurements were made. In each experiment, animals on the −Se diet had significantly lower T_3 production rates than had control animals. Significance of difference from control animals: $^{**}P < 0.005$, $^{***}P < 0.001$.

Strain... / Duration of diet (weeks)	T_3 production (fmol/min per mg of protein)			
	Hooded Lister		Wistar	
	+Se	−Se	+Se	−Se
4	14.5±7.0 (8)	2.0±1.9 (10)**	–	–
6	14.6±3.7 (6)	1.1±2.4 (6)***	14.2±3.7 (6)	1.0±3.7 (6)**

Table. 4. Effect on T_3 production of mixing liver homogenates from Se-deficient (−Se) and control animals (+Se) (the effect of DTT and Na_2SeO_3 is also shown)

Results are means±S.D. for triplicate determinations. Significant changes within groups in T_3 production after the addition of DTT or Na_2SeO_3 are: $^{*}P < 0.05$; $^{**}P < 0.001$.

Addition	T_3 production rate (fmol/min per mg of protein)		
	+Se	+Se/−Se (1:1)	−Se
None	14.3±4.0	10.0±3.0	3.3±2.9
DTT (1 mM)	25.6±1.1***	15.7±4.6	3.6±2.1
Na_2SeO_3			
1 ng of Se/mg of protein	9.0±0.0*	–	< 1.0
10 ng of Se/mg of protein	< 1.0***	–	< 1.0

in Table 3. Marked significant decreases in T_3 production were found in both Wistar and Hooded Lister rats which had consumed the Se-deficient diet. This impaired T_3 production in liver homogenates was apparent after 4 weeks of Se deficiency. In control animals there was no significant difference between rate of T_3 production in frozen livers from Hooded Lister and fresh livers from Wistar rats.

***(b)* Effect of added cofactors.** The addition of NAD, NADP, NADH or NADPH at a final concentration of either 0.62 mM or 6.2 mM to a pooled homogenate of livers from Se-deficient rats (Hooded Lister; 6 weeks) caused no significant increase in T_3 production. Similarly no increase in T_3 production was observed with GSH added at a final concentration of 2.5 mM or 25 mM.

The effect of mixing pooled homogenates obtained from control and Se-deficient animals is shown in Table 4. Mixing homogenates in a 1:1 (v/v) proportion resulted in a T_3 production rate that was not significantly greater than the mean of the production rates for the two homogenates. The inclusion of DTT into the buffer system resulted in a significant activation of T_3 production in incubations of control homogenates, but not in homogenates obtained from Se-deficient animals.

The addition of Se, as selenite, produced no significant increase in T_3 production in Se-deficient animals, and, when added to control animals, T_3 production was inhibited (Table 4).

DISCUSSION

Se deficiency, induced for periods of 4–6 weeks from weaning, produced a marked inhibition of hepatic 5′-deiodination. We were unable to measure 5-deiodinase activity, but the changes we observed in the concentrations of thyroid hormones in the plasma suggests that the activity of this enzyme is also inhibited in Se deficiency.

The plasma concentrations of T_3 and rT_3 are mainly determined by the activities of the hepatic type I 5- and 5′-deiodinase systems [13,14]. If Se deficiency had resulted in an inhibition of only the 5′-deiodinase, plasma T_3 would have fallen, but plasma rT_3 would have increased, as a consequence of impaired catabolism of rT_3 to 3,3′-di-iodothyronine ('T_2'). Alternatively, if Se deficiency inhibited both 5- and 5′-deiodination, then again T_3 would fall but no increase in rT_3 would be observed. Our findings are consistent with the latter explanation.

The fall in plasma T_3, at most 32%, which we observed in our Se-deficient animals was not as great as might be initially expected from our observation that, *in vitro*, hepatic T_3 production was diminished up to 14-fold. However, the circulating concentrations of T_3 and rT_3 result from the balance between their production and catabolism. Thus, if 5- and 5′-deiodinase activity is inhibited in Se deficiency, both production and catabolism of T_3 and rT_3 would be impaired, thus tending to maintain the circulating levels of these hormones within normal limits. This will also result in impaired T_4 utilization and thus increased plasma T_4 concentrations. It is, of course, possible that type II deiodinase activity in non-hepatic tissues or thyroidal synthesis of T_3 and rT_3 may be altered in Se deficiency.

Evidence suggests that the hepatic system responsible for 5- and 5′-deiodination of T_4 is a single microsomal enzyme [21,22]. Cytosolic factors are required for enzymic activity and, in particular, the concentrations of GSH and GSSG are thought to influence 5- and 5′-deiodination [23]. When the ratio GSH/GSSG is high, 5′-deiodination and T_3 production preferentially occur, but when the ratio is low, as may occur in poor nutritional states or non-thyroidal illness, 5-deiodination is activated and 5′-deiodination is inhibited, thus leading to increased rT_3 production [24,25]. Production of T_3 from T_4 may be enhanced *in vitro* by addition of NADPH, which is presumed to act as a substrate for glutathione reductase

and also by the addition of DTT. In our experiments, T_3 production in liver homogenates from Se-adequate rats was activated by DTT, but none of the cofactors tested produced any activation of T_3 production in homogenates obtained from Se-deficient animals. Furthermore, even in severe Se deficiency, liver GSH/GSSG concentrations are not altered, even though there is an increased output of GSH by the Se-deficient hepatocyte [26,27]. Thus Se deficiency is unlikely to have influenced T_3 production by alteration of the redox state of the liver cell, although an effect of changes in the rate of GSH turnover cannot be eliminated.

Our data suggested that the decreased deiodinase activity observed in Se-deficient animals does not result from a lack of essential cofactors, nor from the production of an inhibitor. We used whole homogenates rather than microsomes (microsomal fractions) in our experiments to minimize possible inactivation of the enzyme during preparation and to ensure that all cofactors required for T_3 production would be present. Experiments in which homogenates obtained from Se-deficient animals were mixed with control homogenates demonstrated that liver from Se-deficient animals is probably not lacking in essential cofactors and does not contain potent inhibitors of T_3 production.

It is possible that Se is an essential co-factor for the hepatic 5- and 5'-deiodinase system, but the addition of physiological amounts of Se to incubations of Se-deficient homogenates inhibited rather than stimulated T_3 production. However, these data do not exclude Se as an essential cofactor, since it is likely that Se would need to be incorporated during enzyme synthesis *in vivo* for activity to be restored.

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