Biochem. J. (1990) 266, 743–747 (Printed in Great Britain)

The changes in hepatic enzyme expression caused by selenium deficiency and hypothyroidism in rats are produced by independent mechanisms

Geoffrey J. BECKETT,*‡ Fergus NICOL,† Diane PROUDFOOT,* Keith DYSON,* George LOUCAIDES† and John R. ARTHUR†§

*University Department of Clinical Chemistry, The Royal Infirmary, Edinburgh EH3 9YW, and †Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland, U.K.

Selenium (Se) deficiency for 5 weeks in rats produced changes in the activity of a number of hepatic, renal and plasma enzymes. In animals whose food intake was restricted to 75% of normal for 2 weeks, Se deficiency produced significant increases in the activity of hepatic cytosolic 'malic' enzyme and mitochondrial α-glycerophosphate dehydrogenase (GPD), two enzymes that are particular sensitive to the thyroid-hormone concentrations in tissue. Propylthiouracil-induced hypothyroidism produced significant decreases in 'malic' enzyme and GPD activities. The effect of hypothyroidism on the activity of 'malic' enzyme, GPD and other enzymes studied in liver and plasma was often opposite to that seen in Se deficiency. Glutathione *S*-transferase (GST) activity was increased by both Se deficiency and hypothyroidism, but in hypothyroid animals further significant increases in GST were produced by Se deficiency. These data suggest that the changes in enzyme expression observed in Se deficiency are not caused by decreased tissue exposure to thyroid hormones.

INTRODUCTION

Prolonged selenium (Se) deficiency affects the expression of several hepatic enzymes [1–3]. The activity of Se-dependent glutathione peroxidase (GSH-Px) is markedly decreased, and the activity of other enzymes that are not selenoenzymes, such as cytochrome *c* reductase, may also diminish [1–4]. Certain enzymes, such as the glutathione *S*-transferases (GSTs), which have Se-independent glutathione peroxidase activity, may increase in activity in Se-deficient rats or mice [2,5–7].

The mechanism by which Se deficiency produces changes in hepatic enzyme expression is unknown, but it is thought to be independent of that responsible for the decrease in GSH-Px activity. For example, GST activity can be restored to control values if Se-deficient rats or mice are given low parenteral or dietary doses of Se which are insufficient to produce any significant changes in hepatic GSH-Px activity [2,8,9].

Thyroid-hormone status has many effects on the expression of hepatic enzyme systems. Hypothyroidism induced by propylthiouracil (PTU) in mice is accompanied by an increase in hepatic GST, which has been attributed to an increased half-life of the GST and can be reversed by intraperitoneal injection of 3,3′,5-tri-iodothyronine (T_3) [10]. The hepatic enzymes that are most sensitive to changes in thyroid-hormone status and whose synthesis is decreased in hypothyroidism are mitochondrial α-glycerophosphate dehydrogenase (GPD) and cytosolic 'malic' enzyme [11–13]. The activity of certain enzymes in plasma also changes with thyroid status, and in hypothyroidism plasma creatine kinase (CK) and alkaline phosphatase (ALP) are decreased [14,15]. A decrease in plasma ALP has also been reported in Se-deficient cattle [16].

We have recently described links between thyroid-hormone production and Se status [9,16–18]. Although all thyroxine (T_4) is produced by synthesis *de novo* in the thyroid, more than 90% of the biologically active thyroid-hormone T_3 is produced by 5′-deiodination of T_4 in peripheral tissues. The liver is quantitatively a very important organ for this deiodination reaction (for a review, see [19]). The deiodination of T_4 to T_3 by liver homogenate of Se-deficient rats was less than 10% of that by homogenate from Se-adequate animals [17,18]. These findings led us to suggest that the changes in enzyme expression observed in Se deficiency may result in part from tissue hypothyroidism brought about by impaired hepatic T_3 production [16,20].

In the present study we have measured the changes that occur in the activity of plasma and hepatic enzyme systems in rats with PTU-induced hypothyroidism, Se deficiency or combined hypothyroidism and Se deficiency in order to determine whether they are brought about by a similar mechanism.

MATERIALS AND METHODS

Reagents

Antisera for T_3 and T_4 determinations and anti-rabbit IgG for measurement of TSH were obtained from the

Abbreviations used: T_3, 3,3′,5-tri-iodothyronine; T_4, thyroxine; GSH-Px; Se-dependent glutathione peroxidase; GST, glutathione *S*-transferase; GPD, α-glycerophosphate dehydrogenase; CK, creatine kinase; ALP, alkaline phosphatase; TSH, thyrotropin; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; PTU, propylthiouracil.

‡ To whom correspondence should be addressed.

§ To whom reprint requests should be sent.

Scottish Antibody Production Unit (Carluke, Lanarkshire, Scotland, U.K.). ^{125}I-labelled T_3 and T_4 (sp. radioactivity $< 1200\ \mu Ci/\mu g$) were from Amersham International (Amersham, Bucks., U.K.). Reagents for measurement of plasma TSH were provided by the NIDDK and NIH National Hormone and Pituitary Program, University of Maryland, School of Medicine, MD, U.S.A. All other reagents were from Sigma Chemical Co. or BDH (both of Poole, Dorset, U.K.).

Animals

Weanling male Hooded Lister rats of the Rowett strain were used in all experiments. The animals were individually housed in plastic cages with stainless-steel grid tops and floors; distilled water was available *ad libitum*. Food intake was monitored daily, and body weight was monitored weekly.

Diets

***(a)* Selenium deficiency.** Two groups of animals were fed *ad libitum* for 5 weeks on a synthetic diet [7] containing less than 0.005 mg of Se/kg ('Se-deficient group') or the same diet supplemented with 0.1 mg of Se/kg, as Na_2SeO_3 ('control group'). All diets contained α-tocopheryl acetate (200 mg/kg).

***(b)* Thyroid-hormone deficiency.** To induce hypothyroidism, PTU was given *ad libitum* in the drinking water (0.05% solution) for 5 weeks to groups of rats given the control or Se-deficient diets.

***(c)* Food restriction.** As hypothyroidism results in decreased food intake, two further groups of rats were fed either the Se-supplemented or Se-deficient diet *ad libitum* for 3 weeks, after which the diets were given at 75% of the '*ad libitum*' food intake of animals in group (*a*).

Preparation of plasma and tissue homogenates

Rats were anaesthetized with diethyl ether, and blood was collected by cardiac puncture into heparinized tubes. Plasma was prepared by centrifugation at 1500 ***g*** for 15 min and then stored at −85 °C until required.

Livers were perfused via the portal vein with 150 mmol of KCl/l to remove residual blood. Part of the liver was used immediately for the preparation of mitochondrial and cytosolic fractions. The remainder and the other tissues were frozen in liquid N_2 and stored at −85 °C.

Mitochondria and 105000 ***g*** cytosol were prepared, by differential centrifugation, from a 10% (w/v) homogenate of 1 g of liver in a buffer containing sucrose (250 mmol/l) and Tris/HCl (50 mmol/l), pH 7.5.

Homogenates were firstly centrifuged at 1000 ***g*** for 10 min and the supernatants then centrifuged for 12000 ***g*** for 10 min. The mitochondrial pellet was washed once with homogenization buffer and resuspended in phosphate buffer (125 mmol/l), pH 7.5. The supernatant obtained from the 12000 ***g*** centrifugation was then centrifuged at 105000 ***g*** for 60 min to obtain cytosol.

Plasma measurements

The concentrations of total T_3 and total T_4 in plasma were measured by double-antibody radioimmunoassays [21]. Plasma TSH concentration was measured by double-antibody radioimmunoassay using reagent, standards (rTSH-RP-2) and protocols provided by the NIH National Hormone and Pituitary Program. CK and ALP activities were measured on a Cobas-Bio centrifugal analyser using kit methods (CK-NAC activated; Randox Laboratories Ltd., Crumlin, Co. Antrim, Northern Ireland, U.K.; ALP C-system-a; Boehringer Mannheim Diagnostica, Lewes, Sussex, U.K.).

All assays had an intra-assay coefficient of variation of less than 10%.

Tissue enzyme measurements

GSH-Px activity was measured with H_2O_2 (0.25 mmol/l) as substrate, in the presence of GSH (5 mmol/l) [22].

Cytosolic 'malic' enzyme was determined using $MnCl_2$ (1 mmol/l), malate (0.5 mmol/l) and 45 μM-$NADP^+$ on a Cobas FARA centrifugal analyser, and mitochondrial 'malic' enzyme was assayed as described previously [23].

The activity of GPD in mitochondria and cytosol was measured by using NAD^+-and $NADP^+$-linked reactions as described by Lee & Lardy [23].

The activity of GST was assayed with either 1-chloro-2,4-dinitrobenzene (CDNB) or 1,2-dichloro-4-nitrobenzene (DCNB) as described previously [24].

RESULTS

Plasma hormones and enzymes (Table 1)

Se deficiency produced significant decreases in plasma T_3 and significant increases in plasma T_4 in the animals fed *ad libitum* and also in those that had their food intake restricted. In the two groups of PTU-treated rats plasma T_3 was undetectable. Plasma T_4 concentrations fell to extremely low levels in all animals given PTU, but they were significantly higher in the Se-deficient hypothyroid group than in their Se-adequate counterparts ($P < 0.001$). Restriction of food intake had no significant effect on T_4, T_3 or TSH concentrations, results which we have reported previously [18], but marked increases in plasma TSH concentration were observed in the two groups of animals given PTU.

Both Se deficiency and food restriction produced significant decrease in plasma ALP ($P < 0.01$). Plasma ALP activities in the two PTU-treated groups were significantly lower than in the rats fed *ad libitum* ($P < 0.01$), but no additional effect of Se deficiency was apparent.

Plasma CK activity was significantly ($P < 0.01$) increased by Se deficiency in the control, food-restricted and PTU groups. Food restriction also increased ($P < 0.05$) plasma CK activities in the Se-supplemented group, whereas PTU treatment produced a significant decrease in CK levels in the Se-supplemented, but not in the Se-deficient, group.

Thyroid weights

Thyroid-gland weights were increased in rats given PTU, but were unaffected by Se deficiency or restriction of food intake (Table 1).

Liver enzymes (Table 1)

Hepatic GSH-Px activity was markedly decreased by Se deficiency in control, food-restricted and PTU-treated rats. Food restriction had no effect, whereas PTU administration produced a significant increase in GSH-Px activity.

Se deficiency produced significant increases in GST

Table 1. Thyroid-weight, thyroid-hormone and enzyme measurements in Se deficiency, food restriction and hypothyroidism

Animals (six per group) were fed Se-deficient (Se−), Se-replete (Se+) diets *ad libitum* (control) or at 75% of control food intake (FR). Hypothyroid animals (PTU) received propylthiouracil for the 5 weeks of the study. Significant differences from the 'control Se+' value are shown by superscripts: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.01$ using the paired Student t test. Results are means ± S.D. Abbreviations: Mito, mitochondrial fraction; Cyt, cytosolic fraction; NAD, NAD^+-linked; NADP, $NADP^+$-linked.

Parameter	Control		FR		PTU	
	Se+	Se−	Se+	Se−	Se+	Se−
Thyroid weights (mg)	20 ± 3.9	17.3 ± 1.7	17.4 ± 3.2	16.7 ± 1.9	79.4 ± 13.1[c]	88.3 ± 12.5[c]
Plasma measurements						
T_4 (nmol/l)	88.5 ± 9.6	116.7 ± 8.6[c]	87.8 ± 20.0	120.7 ± 17.1[b]	4.5 ± 0.7[c]	10.2 ± 2.8[c]
T_3 (nmol/l)	1.3 ± 0.28	1.0 ± 0.34[a]	1.1 ± 0.12	0.96 ± 0.19[b]	< 0.1[c]	< 0.1[c]
TSH (ng/ml)	1.1 ± 0.4	1.1 ± 0.2	0.86 ± 0.3	0.83 ± 0.2	37.5 ± 4.3[c]	28.7 ± 6.8[c]
ALP (units/l)	185 ± 17	161 ± 12[b]	155 ± 20[b]	136 ± 13[c]	126 ± 23[c]	126 ± 22[c]
CK (units/l)	152 ± 13	208 ± 45[c]	188 ± 26[b]	254 ± 43[c]	135 ± 30[a]	203 ± 61[c]
Liver measurements						
GSH-PX (units/mg of protein)	1.08 ± 0.16	0.005 ± 0.001[c]	0.98 ± 0.12	0.01 ± 0.002[c]	1.40 ± 0.20[c]	0.02 ± 0.006[c]
GST (CDNB) (μmol/mg of protein)	0.33 ± 0.09	0.54 ± 0.11[c]	0.23 ± 0.06[a]	0.50 ± 0.23[a]	0.69 ± 0.19[c]	0.75 ± 0.27[c]
GST (DCNB) (μmol/mg of protein)	0.032 ± 0.002	0.050 ± 0.004[c]	0.034 ± 0.004	0.052 ± 0.008[c]	0.061 ± 0.004[c]	0.068 ± 0.008[c]
'Malic' enzyme (Cyt) (munits/mg of protein)	57.1 ± 11.9	67.1 ± 9.5	69.1 ± 11.7[c]	92.1 ± 14.1[b]	14.8 ± 3.6[c]	11.6 ± 3.1[c]
'Malic' enzyme (Mito) (munits/mg of protein)	3.3 ± 0.71	3.8 ± 0.6	4.7 ± 0.82[b]	6.8 ± 1.85[c]	1.9 ± 0.64[b]	2.4 ± 0.36[a]
GPD (Mito) (ΔA/min per mg of protein)	0.16 ± 0.04	0.20 ± 0.06	0.13 ± 0.05	0.17 ± 0.03	0.07 ± 0.03[c]	0.15 ± 0.04
GPD (Cyt) (NAD) (ΔA/min per mg of protein)	0.26 ± 0.04	0.32 ± 0.02[a]	0.24 ± 0.01	0.25 ± 0.02	0.26 ± 0.03	0.32 ± 0.05[c]
GPD (Cyt) (NADP) (ΔA/min per mg of protein)	0.11 ± 0.01	0.19 ± 0.02[c]	0.10 ± 0.01	0.13 ± 0.03[a]	0.11 ± 0.01	0.19 ± 0.03[c]
Kidney measurements						
GSH-Px (units/mg of protein)	0.56 ± 0.07	0.03 ± 0.006[c]	0.50 ± 0.07	0.03 ± 0.004[c]	0.76 ± 0.08	0.07 ± 0.01[c]
'Malic' enzyme (Cyt) (munits/mg of protein)	10.0 ± 2.1	14.5 ± 1.5[c]	10.0 ± 3.1	11.3 ± 1.8	7.2 ± 2.2[a]	6.2 ± 1.1[c]

activity, with either CDNB or DCNB as substrate, in control ($P < 0.001$), food-restricted ($P < 0.001$) and PTU-treated animals ($P < 0.05$). Administration of PTU also increased GST activity in both Se-deficient and Se-supplemented rats ($P < 0.001$).

Marked decreases in 'malic' enzyme activity in cytoplasmic and mitochondrial fractions of the liver were produced by PTU. By contrast, the activity of these enzymes increased significantly ($P < 0.01$) in the Se-deficient animals of the food-restricted group, but not in the two other Se-deficient groups.

The activity of GPD in liver mitochondria was significantly decreased by PTU-induced hypothyroidism (Table 1), whereas it was increased by Se deficiency in the food-restricted ($P < 0.05$) and PTU-treated ($P < 0.01$) rats.

Cytosolic GPD activity was significantly increased by Se deficiency ($P < 0.05$), but was not affected by PTU-induced hypothyroidism when measured as the NAD^+- or $NADP^+$-linked reaction.

The cytosolic protein concentration was unchanged by any of the dietary manipulations, the average protein concentration being 10.2 ± 1.9 mg/ml.

Kidney measurements (Table 1)

As in liver, administration of PTU produced significant increases in GSH-Px in kidney cytosol, whereas Se deficiency produced significant decreases in all groups. PTU-induced hypothyroidism caused significant decreases, whereas Se deficiency produced significant increases in cytosolic 'malic' enzyme activity (Table 1).

DISCUSSION

These experiments confirm that Se deficiency can produce significant decreases and increases respectively in plasma T_3 and T_4 concentrations [9,16–18]. In the rats made hypothyroid with oral PTU, plasma T_4 concentrations were greatly decreased, but, despite this, a significant increase in plasma T_4 was still produced by Se deficiency.

Se deficiency and PTU-induced hypothyroidism had opposing effects on plasma CK in that activities were increased by Se deficiency and food restriction and decreased by hypothyroidism. Since CK activity could still be increased by Se deficiency in hypothyroid rats, it appears that different mechanisms are involved in changing plasma CK activity in the two conditions. Both Se deficiency and hypothyroidism decreased plasma ALP activity, which is in accord with our previous findings in plasma of Se-deficient cattle [16].

Se deficiency and PTU-induced hypothyroidism also produced some opposing effects in liver. The activities of 'malic' enzyme in cytosolic and mitochondrial fractions were significantly increased by Se deficiency in the food-restricted animals, but were reduced by hypothyroidism. In the case of mitochondrial and cytoplasmic GPD, Se deficiency tended to increase activities, whereas PTU-induced hypothyroidism caused the expected decrease in mitochondrial GPD activity. The effects of Se deficiency and PTU-induced hypothyroidism on the activity of GSH-Px were also divergent. However, Se deficiency and PTU-induced hypothyroidism both increased hepatic GST activity (determined with either CDNB or DCNB as substrate), and a further significant increase in GST activity could be produced by Se deficiency in hypothyroid animals. Each treatment could therefore be affecting GST induction through similar or different mechanisms, producing additive effects. The results do not distinguish between the two possibilities.

Changes in kidney GSH-Px and cytoplasmic 'malic' enzyme are in accord with those demonstrated in liver.

The fact that hypothyroidism and Se deficiency produce opposite effects on the expression of many hepatic and renal enzymes and on many plasma hormones and enzymes indicates that the changes in enzyme expression that occur in Se deficiency do not result from simple tissue hypothyroidism; this is despite a dramatic decrease in hepatic and renal production of T_3 [9,18]. Although the type I thyroid-hormone deiodinase enzyme system is responsible for production of T_3 through 5′-deiodination of T_4, it also inactivates T_3 by 5-deiodination to produce di-iodothyronines; thus T_3 concentrations in tissue and plasma result from a balance between production and degradation. In Se deficiency the rate of T_3 production in liver homogenates is less than 10% of that found in Se-supplemented rats, but plasma T_3 concentrations remain at approx. 70% of the concentrations found in Se-replete rats [17,18]. This small change in plasma T_3 concentration, despite a large decrease in hepatic T_3 production, indicates that T_3 catabolism is also inhibited by Se deficiency. Hence hepatic GPD and 'malic' enzyme activities are not depressed in Se deficiency as they are by the low cellular T_3 concentrations that occur in hypothyroidism.

It is therefore probable that the changes in some enzyme activities that occur in Se deficiency are not brought about by decreased availability of thyroid hormones.

We thank Mr. A. F. Howie for his help in adapting the 'malic' enzyme assay for use on the centrifugal analyser and Mrs. E. Ward for secretarial assistance.

REFERENCES

1. Reiter, R. & Wendel, A. (1983) Biochem. Pharmacol. **32**, 3063–3067
2. Reiter, R. & Wendel, A. (1984) Biochem. Pharmacol. **33**, 1923–1928
3. Reiter, R. & Wendel, A. (1985) Biochem. Pharmacol. **34**, 2287–2290
4. Hafeman, D. G. & Hoekstra, W. G. (1977) J. Nutr. **107**, 656–665
5. Lawrence, R. A., Parkhill, L. K. & Burk, R. F. (1978) J. Nutr. **108**, 981–987
6. Masukawa, T., Nishimura, T. & Iwata, H. (1984) Biochem. Pharmacol. **33**, 2635–2639
7. Arthur, J. R., Morrice, P. C., Nicol, F., Beddows, S. E., Boyd, R., Hayes, J. D. & Beckett, G. J. (1987) Biochem. J. **248**, 539–544
8. Hill, K. E., Burk, R. F. & Lane, J. M. (1987) J. Nutr. **117**, 99–104
9. Arthur, J. R., Nicol, F., Hutchinson, A. R. & Beckett, G. J. (1990) J. Inorg. Biochem., in the press
10. Williams, M. T., Carrington, H. & Herrera, A. (1986) Biochem. J. **233**, 595–598
11. Torentino, A. C., Richert, D. A. & Westerfeld, W. W. (1966) Biochim. Biophys. Acta **124**, 295–309
12. Dillman, W. H., Schwartz, H. L. & Oppenheimer, J. H. (1978) Biochem. Biophys. Res. Commun. **80**, 259–266
13. Lim, V. S., Henriquez, C., Seo, H., Refetoff, S. & Martino, E. (1980) J. Clin. Invest. **66**, 946–952
14. Doran, G. R. & Wikinson, J. H. (1975) Clin. Chim. Acta **62**, 203–211

15. Hepler, O. E. (1956) Manual of Clinical Laboratory Methods, 4th edn., C. C. Thomas, Springfield
16. Arthur, J. R., Morrice, P. C., Beckett, G. J. (1988) Res. Vet. Sci. **45**, 122–123
17. Beckett, G. J., Beddows, S. E., Morrice, P. C. & Arthur, J. R. (1987) Biochem. J. **248**, 443–447
18. Beckett, G. J., Macdougall, D. A., Nicol, F. & Arthur, J. R. (1989) Biochem. J. **259**, 887–892
19. Leonard, J. L. & Visser, T. J. (1986) in Thyroid Hormone Metabolism (Heinnemann, G., ed.), pp. 189–229, Marcel Dekker, New York and London
20. Arthur, J. R. & Beckett, G. J. (1989) in Selenium in Biology and Medicine (Wendel, A., ed.), pp. 90–95, Springer-Verlag, Heidelberg
21. Ratcliffe, W. A., Challand, G. S. & Ratcliffe, J. G. (1974) Ann. Clin. Biochem. **11**, 224–229
22. Arthur, J. R. & Boyne, R. (1985) Life Sci. **36**, 1569–1575
23. Lee, Y. & Lardy, H. A. (1965) J. Biol. Chem. **240**, 1427–1436
24. Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) J. Biol. Chem. **249**, 7130–7139

Received 9 November 1989; accepted 6 December 1989