Thyroid stimulating hormone and selenium supply interact to regulate selenoenzyme gene expression in thyroid cells (FRTL-5) in culture

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Abstract In the absence of a sodium selenite supplement, FRTL-5 cells showed a reduced activity of cytosolic glutathione peroxidase (cGSH-Px), a marker of selenium status, indicating the cells were Se-deficient. Se-deficient cells showed a 65% reduction in cGSH-Px mRNA abundance but little change in abundance of either phospholipid hydroperoxide glutathione peroxidase or type 1 deiodinase (IDI) mRNA. In Se-replete cells increased thyroid stimulating hormone (TSH) caused a small decrease in IDI abundance but in Se-deficient cells TSH caused a large increase. The results indicate an interaction between TSH and Se status in the regulation of thyroid selenoenzyme synthesis.

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Key words: Selenium; Deiodinase; Thyroid; Messenger RNA; 3′ Untranslated region; Thyroid stimulating hormone

1. Introduction

The micronutrient selenium, which is essential for both man and animals [1,2], is present as selenocysteine (Se-Cys) in at least 30 proteins: these include cytosolic glutathione peroxidase (cGSH-Px) and phospholipid hydroperoxide glutathione peroxidase (PHGSH-Px), which have been proposed to protect cells from oxidant damage [3], and type 1 iodothyronine deiodinase (IDI) which is involved in thyroid hormone metabolism [4–6].

Thyroid hormones are produced by the thyroid in the relatively inactive form thyroxine (T_4) and converted to the biologically active 3,3′,5-triiodothyronine (T_3) by deiodination. T_4 and T_3 synthesis is controlled by thyroid stimulating hormone (TSH), secreted by the pituitary gland in response to circulating levels of the thyroid hormones and by thyroidal autoregulatory mechanisms in response to iodine availability. In addition, the proportion of thyroid hormones present as active T_3 circulating in the blood is determined by the activity of the deiodinating enzyme, IDI, in the thyroid and peripheral tissues. The activity of tissue IDI is altered by the amount of Se available in the diet [4–6]. The thyroid cell line FRTL-5 [7] expresses IDI and this is modulated by TSH, growth factors and the cytokine tumour necrosis factor-α [8,9].

There is complex regulation of the synthesis of cGSH-Px, PHGSH-Px and IDI as a result of changes in dietary Se supply: there are differences both within a given tissue and also between tissues in the response of the activity of each enzyme, and the abundance of their mRNAs [10]. In both the liver and thyroid cGSH-Px is most affected by Se deficiency whereas of the three enzymes PHGSH-Px is least affected in the liver with only a 75% decrease in activity and no change in mRNA abundance. In contrast, IDI activity and mRNA abundance increase in the thyroid. It appears that the differential regulation between liver and thyroid is physiologically important in that it allows, under conditions of limiting Se supply, a preferential maintenance of particular selenoenzymes in specific tissues. The aim of the present work was to determine if the thyroid-specific differences in response to Se are maintained in cell culture and whether responses to altered TSH concentrations may underlie this tissue specificity.

2. Material and methods

2.1. Cell culture

FRTL-5 cells [7], a continuous line of epithelial cells derived from normal rat thyroid (American Type Culture Collection, Rockville, MD, CRL 8305) were maintained in Coon's modified Ham's F-12 medium supplemented with 5% newborn calf serum, penicillin (100 U/ml)/streptomycin (100 mg/ml), L-glutamine (2 mmol), fungizone (1.25 mg/ml), bovine TSH (1 mU/ml) and a hormone mixture containing somatostatin (10 ng/ml), glycyl-L-lysine acetate (10 ng/ml), hydrocortisone (10 mM), insulin (10 mg/ml), and transferrin (5 mg/ml). This constituted the normal medium.

In order to induce Se depletion the cells were grown in similar medium but with only 0.5% serum and with no supplementation of Se. To maintain cells in a Se-replete condition they were cultivated in medium containing 0.5% serum but supplemented with 7.5 ng/ml sodium selenite. Medium was changed every 2 days.

In order to study the interaction between Se and TSH, the cells were grown in Se-replete medium and Se-deficient medium containing the hormone mixture modified so as to contain 0.5 mU/ml, 1 mU/ml, 2 mU/ml or 4 mU/ml TSH.

2.2. cGSH-Px activity and protein estimation

cGSH-Px enzyme activity was measured in cells which had been washed, resuspended and homogenised in phosphate-buffered saline, pH 7.4. Aliquots of cell homogenates were incubated with 0.1% peroxide-free Triton X-100, 0.3 mM NADPH, 5 mM reduced glutathione and 0.7 U/ml glutathione reductase. Oxidation of NADPH was followed spectrophotometrically at 340 nm after addition of 2.2 mM H_2O_2 to the samples [11]. Total protein was measured according to Bradford's procedure [12].

2.3. DNA probes and chemicals

The cGSH-Px probe, a gift from Dr P.R. Harrison, Beatson Institute, Glasgow, was a 0.7-kb *Eco*RI fragment from the 1.5-kb cDNA of the mouse gene cloned into pUC12 [13]. The IDI probe was a 0.55-kb *Hin*dIII fragment of the rat cDNA, a gift from Dr M. Berry and Dr P.R. Larsen, Harvard Medical School, Boston, MA, USA [14]. The PHGSH-Px probe [15] was a 0.8-kb *Eco*RI restriction fragment of the rat cDNA obtained from Dr R. Sunde, University of Missouri-Colombia, USA. The 18S rRNA cDNA [16] was obtained from Dr R. Fulton, Beatson Institute, Glasgow, UK, and a 1.4-kb *Bam*HI fragment derived from it was used for hybridisation.

Multiprime labelling kits, Hyperfilm-MP and [^{32}P]dCTP were purchased from Amersham International, Amersham, Bucks., UK. Genescreen nylon membrane was purchased from NEN-Dupont, and others chemicals were of either Analar or molecular biology grade.

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2.4. RNA extraction and hybridisation analysis

Total RNA was extracted by the guanidinium-phenol-chloroform extraction method [17] and its purity assessed by the A_{260}/A_{280} absorbance ratio. RNA species were then separated by electrophoresis through a denaturing 2.2 M formaldehyde, 1.2% w/v agarose gel [18] and transferred to nylon membrane (Genescreen from NEN Dupont) by capillary blotting. RNA was fixed to the membrane by exposure to UV light and the membranes were stored dry until required.

Hybridisation assays were carried out as described previously [19]. Membranes were prehybridised overnight at 42°C with 0.1 mg/ml denatured salmon sperm DNA in buffer containing 50% formamide, 10% dextran sulphate, 0.2% BSA, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.1% sodium pyrophosphate, 1% SDS and 50 mM Tris-HCl, pH 7.5. 50 ng DNA probe was labelled with [^{32}P]dCTP by random priming (Multiprime kit) and the labelled DNA was separated from free nucleotides by gel filtration on Sephadex G-50; probe specific radioactivities were approximately 10^9 cpm/mg of DNA. The labelled probes were added to the prehybridisation mix and hybridised at 42°C for 16 h. The membranes were washed to remove non-specifically bound probe as follows: for the cGSH-Px and IDI probes two washes in 2×SSC (1×SSC = 0.15 M NaCl/0.015 M sodium citrate) at room temperature for 5 min, followed by two washes at 65°C for 1 h in 1×SSC/1% SDS and a final wash in 0.1×SSC at room temperature; for the PHGSH-Px probe two washes in 2×SSC at room temperature for 5 min, followed by two washes at 65°C for 30 min in 1×SSC/1%SDS and a final wash in 0.1×SSC at room temperature. After hybridisation with the 18S rRNA probe the filter was washed twice in 2×SSC at room temperature for 5 min, twice at 65°C for 1 h in 0.2×SSC/1% SDS and then finally once with 0.1×SSC at room temperature. Specific hybridisation was then detected and quantified by direct imaging using a Canberra Packard Instantimager. After analysis membranes were washed in 0.1% SDS for 10 min at 95°C before rehybridisation to other probes.

2.5. Data analysis and statistics

The radioactivity of each band after hybridisation with each DNA probe was measured using the Instantimager. The radioactivity in defined areas corresponding to the bands was corrected for background and expressed as a ratio to the equivalent values from the 18S ribosomal RNA hybridisations. This allowed correction for small variations in the amount of total sample RNA loaded and resulted in the mRNA abundance being expressed per unit of ribosomal RNA. The mRNA abundances per unit mRNA were then expressed as percentages of the mean value for the Se-replete cells on each filter; this enables direct comparison between filters. Finally, the data were analysed using a Mann-Whitney *U*-test; $***P < 0.001$, $**P < 0.01$, $*P < 0.05$.

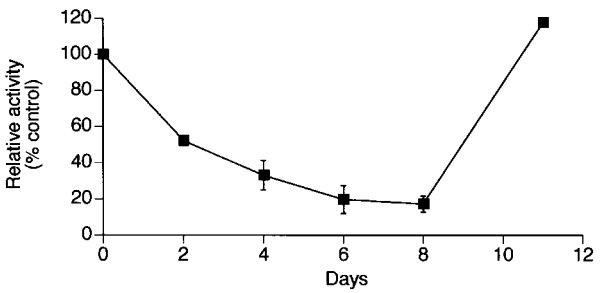

Fig. 1. The effect of Se depletion and repletion on cGSH-Px activity in FRTL-5 cells. Cells were maintained either in control medium with a supplement of 0.5% serum, hormone mixture and 7.5 ng/ml sodium selenite or in medium supplemented with 0.5% serum and hormone mixture but without a supplement of sodium selenite for 8 days and then returned to control medium. cGSH-Px activity is expressed as a percentage of activity found in FRTL-5 cells maintained in control Se-replete medium (mean activity ± S.E.M. = 10.3 ± 1.2 mU/mg protein). Results are reported as the mean ± S.E.M. of four samples independently processed in two separate experiments for days 4, 6 and 8 ($n = 4$). At days 2 and 11, two samples were processed and showed similar activity; the mean value is reported.

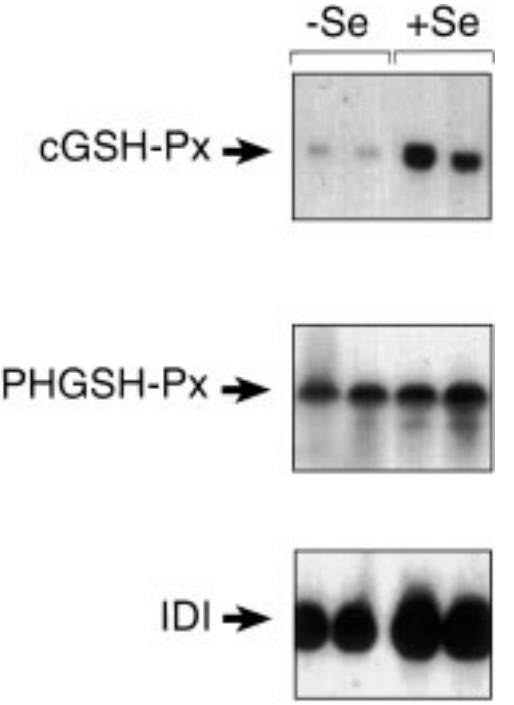

Fig. 2. Northern hybridisation of total FRTL-5 RNA showing the effect of Se deficiency on cGSH-Px, PHGSH-Px and IDI expression. All lanes were loaded with 20 μg of total RNA and filters were hybridised successively with probes specific to cGSH-Px, PHGSH-Px and IDI. Results show the level of the three corresponding RNAs for duplicate samples detected by autoradiography. Note the considerable reduction in cGSH-Px mRNA abundance compared to the small effect on PHGSH-Px and IDI respectively.

3. Results

3.1. Enzymatic activity

Culturing FRTL-5 cells in Se-deficient medium caused a decrease in cGSH-Px activity compared to cells maintained in Se-replete medium (mean activity ± S.E.M. = 10.3 ± 1.2 mU/mg protein). There was a 67% decrease in cGSH-Px activity after 4 days, a 80% decrease after 6 days and a 83% decrease after 8 days (see Fig. 1). When the Se-deficient FRTL-5 cells were subsequently placed in a medium containing 7.5 ng/ml sodium selenite an increase in cGSH-Px activity occurred, reaching 118% of the original cGSH-Px activity within 3 days (day 11).

3.2. Selenoenzyme gene expression during Se deficiency

Visual inspection of both autoradiographs and images from the Instantimager showed that after 8 days culture in Se-deficient medium the cGSH-Px mRNA abundance in FRTL-5 cells was greatly reduced, whereas the PHGSH-Px and IDI mRNA abundances showed little change (Fig. 2). The mean cGSH-Px mRNA abundance in Se-deficient FRTL-5 cells was significantly decreased by 65 ± 7% ($P < 0.001$) compared with the abundance in cells cultivated in a Se-replete medium; in contrast there were no statistically significant changes in the abundance of either PHGSH-Px or IDI mRNA: a decrease of 19 ± 7% and 31 ± 10% respectively compared with the control cells. These effects of Se deficiency on selenoenzyme mRNAs in cell culture were different, in absolute terms, from those observed previously on selenoenzyme gene expression in the thyroid in vivo [10].

3.3. Effect of TSH on selenoenzyme gene expression

In order to investigate whether this difference was due to an influence of TSH in vivo the effects of TSH were investigated in cells grown under Se-replete and Se-deficient conditions. Cells were grown in medium containing different TSH concentrations, both higher and lower than 1 mU/ml; the normal circulating TSH levels in the rat are around 0.2 mU/ml [20] but both rat FRTL-5 cells and human thyrocytes require higher concentrations of bovine TSH (~1–10 mU/ml) in cell culture [7,9,21]. The results are shown (Figs. 3 and 4) as the values obtained in the Se-deficient medium or the Se-replete medium as a percentage of the value obtained in cells cultured in 1 mU/ml TSH. Under Se-replete conditions increasing the TSH concentration in the medium (Fig. 3) from 1 mU/ml to 4 mU/ml had no significant effect on the abundance of cGSH-Px or PHGSH-Px mRNAs whereas the IDI mRNA abundance was decreased by 36% ($P < 0.01$). Under Se-deficient conditions increasing the TSH concentration in the medium (Fig. 3) again had no effect on the PHGSH-Px mRNA abundance whereas it caused a significant 111–135% increase in IDI mRNA abundance; the effect on cGSH-Px was inconsistent, 2 mU/ml TSH causing a 36% decrease ($P < 0.05$) in mRNA abundance and 4 mU/ml causing no significant change.

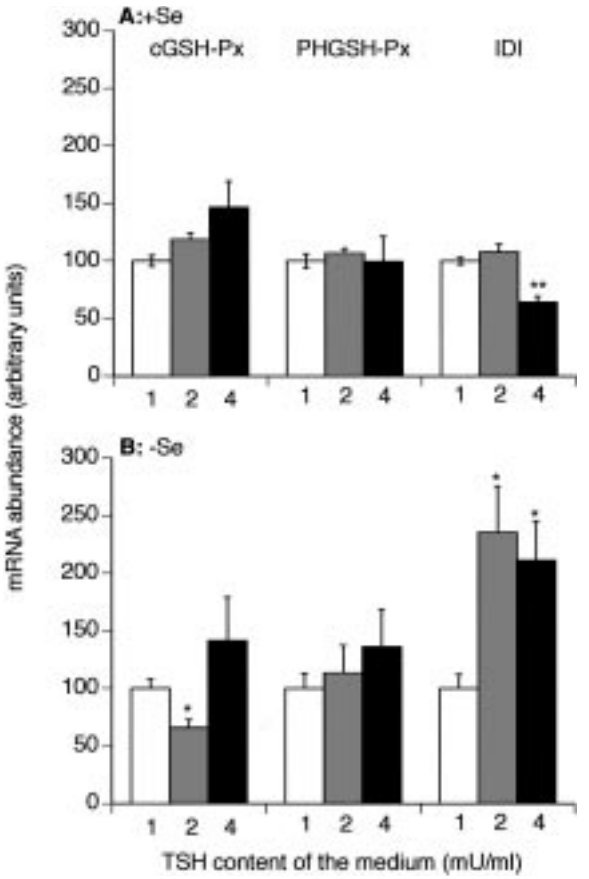

Fig. 3. The effect of increased TSH on selenoenzyme mRNA abundance in FRTL-5 cells. All RNA samples were analysed by Northern hybridisation under identical conditions; RNA samples were loaded so as to allow direct comparison of mRNA abundances at different TSH concentrations under either Se-replete or Se-deficient conditions. Filters were hybridised successively with probes for the three selenoenzymes mRNAs and finally for 18S RNA. Results are expressed in arbitrary units as a percentage of mRNA abundance in the cells grown in 1 mU/ml TSH. Results are means ± S.E.M. ($n = 6$) from two experiments. Data were analysed by a Mann-Whitney U-test (*$P < 0.05$; **$P < 0.01$, compared to the 1 mU/ml group).

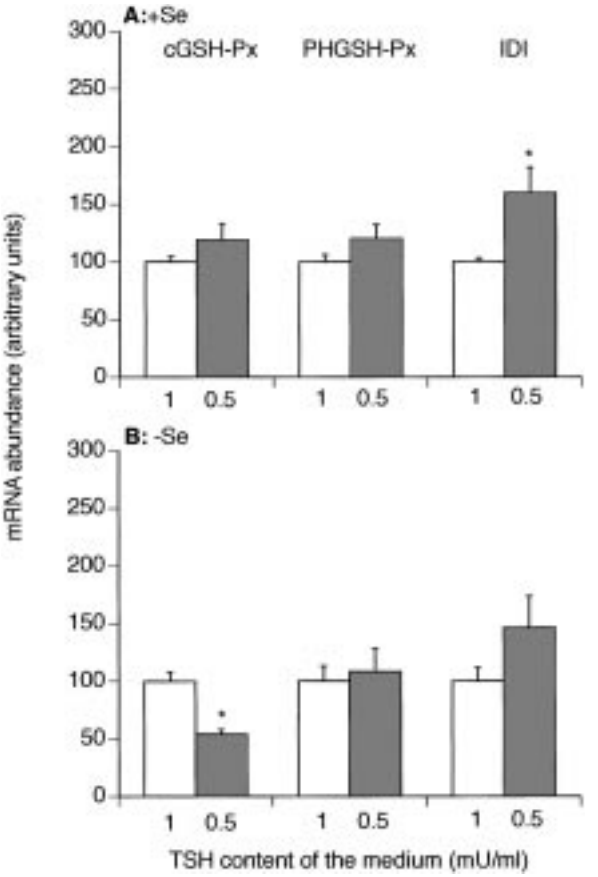

Fig. 4. The effect of decreased TSH on selenoenzyme mRNA abundance in FRTL-5 cells. All RNA samples were analysed by Northern hybridisation under identical conditions; RNA samples were loaded so as to allow direct comparison of mRNA abundances at different TSH concentrations under either Se-replete or Se-deficient conditions. Filters were hybridised successively with probes for the three selenoenzymes mRNAs and finally for 18S RNA. Results are expressed in arbitrary units as a percentage of mRNA abundance in the cells grown in 1 mU/ml TSH. Results are means ± S.E.M. ($n = 6$) from two experiments. Data were analysed by a Mann-Whitney U-test (*$P < 0.05$, compared to the 1 mU/ml group).

Decreased TSH (Fig. 4) had no effect on cGSH-Px or PHGSH-Px mRNA abundances under Se-replete conditions but in Se-deficient cells it induced a 47% decrease ($P < 0.05$) in cGSH-Px mRNA. The abundance of the IDI mRNA was significantly increased by the lower TSH concentration under Se-replete conditions but in Se-deficient conditions there was no statistically significant change.

4. Discussion

The present data demonstrate that the thyroid FRTL-5 cell line is sensitive to Se supply and responds to Se depletion and repletion with decreased and then restored cGSH-Px activity, considered as an indicator of overall Se status [22]. This is similar to the situation in vivo where thyroid cGSH-Px activity is lowered by Se deficiency [10]. However, the response of cGSH-Px, PHGSH-Px and IDI mRNA abundance to Se de-

pletion shows distinct differences in the FRTL-5 cells compared with the thyroid in vivo. In the present cell culture model cGSH-Px mRNA abundance is decreased whereas PHGSH-Px and IDI mRNAs are not altered and this is in contrast with the unchanged cGSH-Px mRNA abundance and the increase of both PHGSH-Px and IDI mRNA abundances in vivo. It is clear therefore that the response of thyroid cells to Se deficiency is different in absolute terms in vitro compared with in vivo; however, the hierarchy of changes in selenoenzymes remains the same.

An influence of TSH on thyroid selenoenzyme expression is one possible explanation of the difference between the effects of Se deficiency on expression in vivo and in vitro. Indeed, the present data show that when FRTL-5 cells were cultured in media containing different amounts of TSH the mRNA abundances of both the cGSH-Px and IDI were modified whereas the mRNA abundance of the PHGSH-Px remained unchanged (Fig. 3). The response to TSH depended on the Se status of the cells, with for example the IDI mRNA showing a large significant increase in abundance with increased TSH under Se-deficient conditions and an opposite decrease in abundance under Se-replete conditions. Clearly in this model system the gene expression of IDI is modulated by the amount of TSH present in the medium during Se deficiency. This extends the previous observation that in cultured thyrocytes TSH alters IDI mRNA abundance [8,9] and enzymatic activity [9,21]. Circulating TSH is increased by up to 2-fold in severely Se-deficient rats [20] and the present data suggest that modulation of IDI and cGSH-Px expression by TSH may explain the increased IDI mRNA abundance in the thyroid during Se deficiency in vivo.

The relative effect of Se deficiency on selenoenzyme mRNA abundances in FRTL-5 cells (Fig. 2; cGSH-Px $\gg$ PHGSH-Px $\cong$ IDI) is similar to that seen in liver and in hepatoma cells where the hierarchy of selenoenzyme response to limited Se supply appears related to the translational mechanism for insertion of Se into selenoenzymes [10,23]. The different selenoenzyme 3′ UTR sequences, which are involved in the incorporation of Se-Cys into selenoenzymes, determine the efficiency of translation of the different mRNAs [14,24–27] and their different sensitivity to Se depletion in transfected hepatoma cells [28]. Thus, the 3′ UTR from PHGSH-Px can incorporate Se-Cys more effectively than cGSH-Px 3′ UTR under conditions of low Se supply [23], leading to differential stability and translation of the two mRNAs [23,28]. It is likely therefore that in thyroid cells in culture, as in hepatoma cells and liver, PHGSH-Px and IDI are preserved more than cGSH-Px partly as a result of different sensitivity of the 3′ UTR-based Se-Cys incorporation mechanism and mRNA stability/translation to Se supply. Furthermore, since the relative effect of Se deficiency on the expression of the three selenoenzyme mRNAs in FTRL-5 cells is similar to that in the thyroid, it is likely that this mechanism partly underlies the different effects seen on selenoenzymes in vivo. The effects of Se deficiency on the thyroid in vivo appear to represent the effects of TSH superimposed on the channelling of the available Se by the 3′ UTRs during translation of the mRNAs.

In conclusion, the present data show that both TSH and Se deficiency induce changes in selenoenzyme gene expression in FRTL-5 cells in culture; furthermore the effects of TSH depend on the Se status of the cells. The data suggest that the effects of Se deficiency on thyroid gene expression in vivo depend upon several interacting factors, and that the thyroid-specific nature of these effects is partly due to effects of circulating TSH concentrations found in Se deficiency.

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