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Oxidant/Antioxidant Status in Relation to Thyroid Hormone Metabolism in Selenium- and/or Iodine-Deficient Rats

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Iodine and selenium are essential components of normal thyroid hormone metabolism and are involved in the modulation of antioxidant defense system. This study was designed to evaluate the extent of peroxidation of lipids and activities of antioxidant enzymes (AOEs) in various tissues of iodine- and/or selenium-deficient rats in relation to thyroid hormone metabolism. Iodine deficiency caused marked enhancements in glutathione peroxidase (GSHPx), superoxide dismutase (SOD), and catalase (CAT) activities in thyroid but did not cause lipid peroxidation (LP), indicating the occurrence of an adaptive response that protected the gland against oxidative stress induced by high levels of thyroid stimulating hormone (TSH). Except significant reduction in CAT activity in liver and kidney and an enhancement of SOD in kidney, iodine deficiency did not cause any other alterations in other tissues. Selenium deficiency and combined iodine and selenium deficiency caused significant alterations in AOE activities in all tissues and caused significantly high levels of LP in thyroid, liver, brain, and plasma, but not in kidney. Alterations in selenium-involved deficiencies appeared to be mainly caused by substantial losses of GSHPx activity; however, compensatory changes in SOD and CAT activities were also observed. J. Trace Elem. Exp. Med. 17:109–121, 2004. © 2004 Wiley-Liss, Inc.

Key words: selenium; iodine; lipid peroxidation; glutathione peroxidase; superoxide dismutase; catalase

INTRODUCTION

Thyroid hormones are essential for the maintenance of normal metabolic function in living organisms and regulate and promote cellular growth and development. Iodine is a structural component and, thus, a primary requirement

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for thyroid hormone synthesis and regulation [1,2]. The prohormone thyroxine (T_4) is produced exclusively in the thyroid gland. T_4 in the circulation enters all tissues of the body, where it may be converted to the metabolically active hormone 3,5,5′-triiodothyronine (T_3) by isozymes of iodothyronine 5′-deiodinase, which are characterized by their tissue distribution, structure, and biochemical properties [1,2]. Selenium also is essential for appropriate thyroid hormone synthesis, activation, and metabolism because the three isozymes of iodothyronine 5′-deiodinase are selenoenzymes [3–5].

Biosynthesis of thyroid hormones involves thyroidal trapping of serum iodide (iodide transport), incorporation of iodine into tyrosine, coupling of iodinated tyrosyl residues of thyroglobulin, and proteolytic cleavage of follicular thyroglobulin to release the iodothyronines [2,6]. Thyroid follicles produce H_2O_2 as a substrate of thyroperoxidase continuously throughout life because during the thyroid hormone synthesis the iodination of tyrosyl residues on thyroglobulin requires the generation of H_2O_2 in high concentration [1,2,7]. Therefore, thyrocytes need a very potent antioxidative defense system against H_2O_2 and reactive oxygen intermediates derived thereof, and an effective cell defense system is essential for the maintenance of normal thyroid function and protection of the gland. In fact, the thyroid cell, as are other cells, is protected by the family of glutathione peroxidase enzymes (GSHPx), superoxide dismutase (SOD), and catalase (CAT). GSHPx neutralizes cytotoxic H_2O_2 and its oxidative by-products [8]. Selenium, thus, plays another important role in the thyroid gland through selenium-dependent GSHPx. However, the relations between selenium and thyroid function are complex and dual. On the one hand, being an integral component of GSHPx and thus as an essential part of the antioxidant defense system, selenium protects the cells from oxidative stress. In case of iodine deficiency, thyroid cells are stimulated by high levels of thyroid-stimulating hormone (TSH) to produce excessive amounts of H_2O_2. When selenium deficiency accompanies to severe iodine deficiency, it may increase the damage in thyroid tissue because of loss of protection from toxic level of H_2O_2 and peroxidative stress because in low selenium conditions less GSHPx is expressed in thyrocytes. On the other hand, being an active component of iodothyronine deiodinases, selenium is involved in thyroid hormone metabolism and, thus, may spare iodine by decreasing the catabolism of prohormone T_4 when a shortage of iodine intake exists [9,10]. Therefore, it is crucial to maintain the optimum levels of the two elements.

Although there are several reports suggesting the modulation of the antioxidant defense system by the thyroid state and the influence of thyroid hormones on the mitochondrial antioxidant capacity, the available data deal with limited tissues [11–16]. In addition, the effects of selenium deficiency and particularly the combined iodine and selenium deficiency have not been studied in detail with respect to oxidant and antioxidant status in various tissues. This study, therefore, was conducted to elaborate possible alterations of antioxidant enzyme (AOE) activities and extent of lipid peroxidation (LP) in various tissues, including thyroid, liver, kidney, brain, and plasma/erythrocytes of rats in three different types of trace element deficiency states (iodine and/or selenium deficiency) in relation to thyroid hormone metabolism.

MATERIALS AND METHODS

Materials

Commercial kits for TSH, total T_4 (TT_4), and total T_3 (TT_3), as well as for free forms (FT_4, FT_3) were purchased from Bechman-Coulter (Immunotech), France. All other chemicals were from Sigma Chemical Co (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

Animals and Diets

Three-week-old male Wistar rats (54.3 ± 1.8 g) supplied by RFFA Credo Animal Breeding Center (Saint Germain, France) were used in all experiments. The animals were housed as a group in plastic cages with stainless-steel grid tops and the cages were placed in a room with controlled temperature (23 ± 2°C), humidity (50%) and a 12-h light-dark cycle. Body weight (bw) was monitored weekly. Feeding period was 5 weeks, six animals were used for each four groups and animals received ad libitum diet and water.

Experimental groups: 1) Control group (C) was fed with Torula yeast-based selenium-deficient diet supplemented with 0.1 mg selenium/kg and drinking water. 2) Iodine-deficient and selenium normal group (ID) received the same diet and 1% sodium perchlorate-containing water. 3) Iodine normal and selenium-deficient group (SeD) were fed with Torula yeast-based selenium-deficient diet containing less than 0.005 mg of selenium/kg and received regular drinking water. 4) Iodine and selenium-deficient group (ISeD) received both selenium-deficient diet and 1% sodium perchlorate-containing water. A second control group was pair-fed according to the food consumption observed in ISeD group.

Preparation of Plasma and Tissue Homogenates

All animals were killed by decapitation under nembutal anesthesia at the end of feeding period. Venous blood samples were collected into heparinized tubes after decapitation of rats anaesthetized. Plasma and erythrocyte (red blood cells [RBCs]) were separated after centrifugation at 800 g for 15 min. All blood samples were immediately aliquoted and stored in freezer at −80°C until analysis. Thyroid gland, liver, brain, and kidney tissues were removed and weighted. All tissues were frozen immediately in liquid nitrogen and stored at −80°C. It was necessary to pool two thyroid samples in each group to obtain sufficient material for all analysis. Tissue homogenates used for determining AOE (GSHPx, SOD, CAT) activities and thiobarbituric acid reactive substances (TBARS) concentrations were prepared in a volume of ice-cold buffer containing tris (10 mM), diethylenetriaminepentaacetic acid (1 mM), and phenylmethanesulphonyl fluoride (1 mM; adjusted to pH 7.4) using a Teflon pestle homogenizer to obtain 10% (w/v) whole homogenate.

Measurement of Thyroid Hormones

Thyroid hormone status was determined by measuring the plasma TSH, TT_4, TT_3, and FT_4, FT_3 concentrations by radioimmunoassay using commercial kits supplied by Bechman-Coulter (Immunotech), France.

Measurement of AOEs and LP Levels

The activity of GSHPx was determined by measuring spectrophotometrically the decrease in NADPH concentration, which is proportional to the enzyme, by using t-butyl hydroperoxide as the substrate [17]. One unit of GSHPx activity is defined as the amount of GSHPx required to oxidize 1 μmol of NADPH per minute. The specific activity was expressed in units per mg protein for tissues, and in units per g hemoglobin for RBCs. The SOD activity was determined by monitoring the auto-oxidation of pyrogallol at 420 nm [18]. One unit of total SOD activity is defined as the amount of enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%. The activity of CAT was measured spectrophotometrically at 240 nm as the decomposition of H_2O_2 [19]. One unit of CAT activity is defined as the amount of enzyme required to decompose 1 μmol of H_2O_2 in 1 min. The specific activity for SOD and CAT was expressed in units per mg protein for tissues, and in units per mg hemoglobin for RBCs. LP levels were quantified by measuring TBARS concentrations by spectrofluorometric assay described by Richard et al. [20]. TBARS is expressed as nanomoles of malondialdehyde per mg protein for tissues and as nanomoles of malondialdehyde per liter for plasma. The protein content in the samples was determined by the method of Lowry [21]. Selenium concentrations in liver, brain, and kidney were determined by a method of gas chromatography-mass spectrometry using the isotopic dilution technique [22]. Plasma concentrations of selenium were determined by electrothermal atomic absorption spectrometry (Model 3030, Perkin Elmer with a Zeeman background correction, a HGA 600 graphite furnace). Standard Reference Material [NIST-NBS-1577b] (bovine liver) was used for quality control of selenium analysis.

Statistical Analysis

Experimental data were analyzed with one-way analysis of variance (ANOVA) followed by Student's *t*-test. Kruskal-Wallis and Mann-Whitney *U* test were used for parameters measured in thyroid tissue. A P value of 0.05 was considered significant. Values are given as mean ± SD.

RESULTS

Thyroid Weights

Iodine deficiency produced a significant increase in thyroid size and weight. Relative thyroid weight (C: 9.84, ID: 21.3, SeD: 9.1, and ISeD: 23.91 mg thyroid /100 g bw) did not change in SeD rats but significantly increased in ID (117%), and in ISeD rats (~140%).

Thyroid Hormones and Selenium Levels

Iodine deficiency caused a significant increase in plasma TSH and lowered the levels of TT_4 and TT_3. Selenium deficiency did not cause any alteration in TSH

TABLE I. Thyroid Hormone Parameters in Selenium- and/or Iodine-Deficient Rats

Group	TSH (ng/mL)	TT_4 (nmol/L)	TT_3 (nmol/L)
C (n = 6)	3.2 ± 0.7[a]	57.7 ± 3.9[a]	0.97 ± 0.08[a]
ID (n = 6)	29.3 ± 8.4[b]	13.3 ± 1.2[b]	0.57 ± 0.05[b]
SeD (n = 6)	3.7 ± 1.1[a]	73.2 ± 11.5[c]	0.85 ± 0.08[c]
ISeD (n = 6)	41.0 ± 2.0[c]	18.3 ± 5.3[d]	0.87 ± 0.31[ac]

[a,b,c,d] Values in columns not sharing a common superscript differ significantly, $P < 0.05$. Values are given as mean ± SD.

TABLE II. Selenium Levels in Various Tissues and Plasma of Selenium- and/or Iodine-Deficient Rats

Tissue	Group	Liver	Brain	Kidney	Plasma*
Se (μg/g tissue)	C (n = 6)	0.6 ± 0.10[a]	0.13 ± 0.008[a]	0.8 ± 0.08[a]	3.91 ± 0.45[a]
	ID (n = 6)	0.8 ± 0.07[a]	0.14 ± 0.009[a]	0.8 ± 0.04[a]	3.74 ± 0.22[a]
	SeD (n = 6)	0.02 ± 0.01[b]	0.09 ± 0.005[b]	0.13 ± 0.02[b]	0.11 ± 0.03[b]
	ISeD (n = 6)	0.03 ± 0.01[b]	0.10 ± 0.022[b]	0.16 ± 0.05[b]	0.16 ± 0.02[b]

[a,b] Values in columns not sharing a common superscript differ significantly, $P < 0.05$. Values are given as mean ± SD.
* Selenium concentrations in plasma are given as μg/L.

level but increased the TT_4 and decreased the TT_3 concentrations. The highest TSH concentrations occurred in ISeD rats. TT_4 and TT_3 were lower than the control values, but higher than those of ID rats (Table I). (Since the same trend was observed for FT_4 and FT_3, only the total values are included in the text.)

Selenium concentrations were found to be substantially low (> 96%) in liver, kidney, and plasma of SeD and ISeD rats. But the decrease in brain was only ~35% reflecting a better protection (Table II).

AOEs and LP Levels

Iodine deficiency did not cause any modification in LP in any tissue but, in SeD and ISeD rats, high levels of LP were observed in all tissues, except kidney (Figs. 1–5). Thyroidal AOE activities were affected by all deficiency states (Fig. 1). GSHPx decreased in SeD, but increased by ID (150%) and ISeD (~40%). The activity of thyroidal CAT increased in all deficiency states and a significant increase in SOD activity was observed only in ISeD.

In other tissues of ID rats, the only alterations observed were higher SOD activity in kidney, and lower CAT activity in kidney and liver. GSHPx was markedly low in all tissues in SeD and ISeD rats. SOD increased in all deficiency states and CAT decreased in ID and ISeD in kidney, whereas SOD increased only in ISeD and CAT decreased in all deficiency states in liver.

There was no change other than marked GSHPx losses and LP enhancement in SeD and ISeD in brain. The same was true for plasma LP and GSHPx in RBC. In addition, CAT activity of RBC decreased in SeD and ISeD (Fig. 5). The

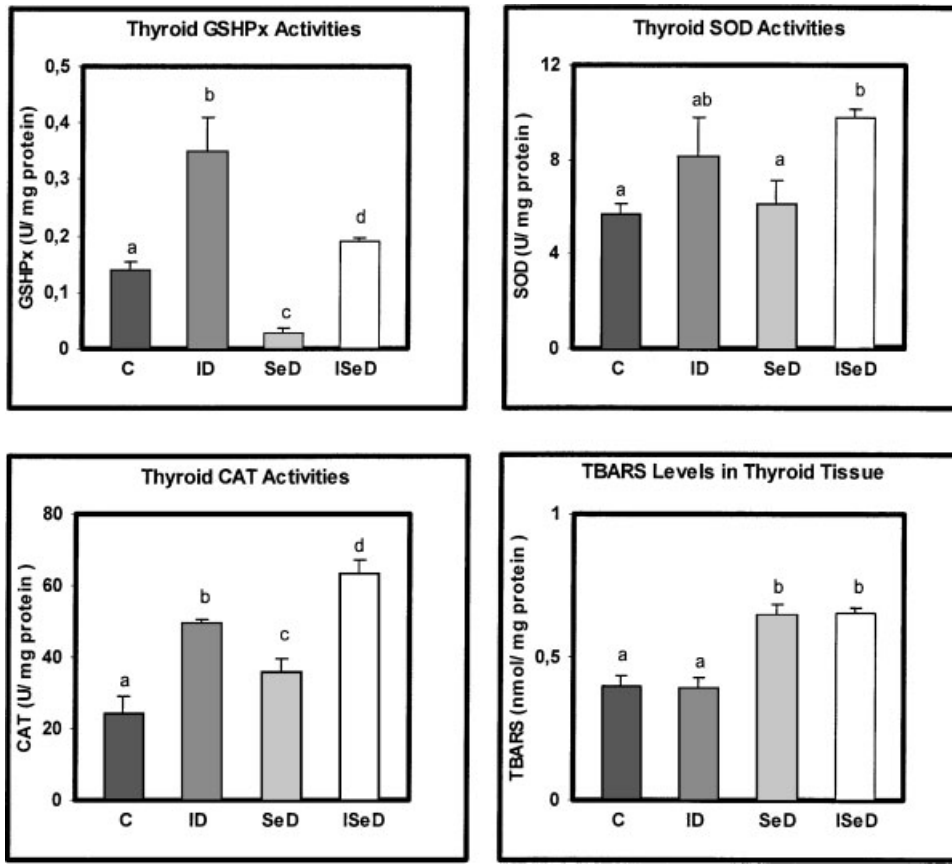

Fig. 1. AOEs and LP levels in thyroid tissues of selenium- and/or iodine-deficient rats. Superscripts of different letters differ significantly ($P < 0.05$) from each other. C, control group; ID, iodine-deficient and selenium-normal group; SeD, iodine-normal and selenium-deficient group; ISeD, iodine- and selenium-deficient group; GSHPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; TBARS, thiobarbituric acid-reactive substances.

control pair-fed group did not differ from the freely fed control group for all measured parameters (data not shown).

DISCUSSION

Iodine deficiency and resulting hypothyroidism was evidenced by increased weights (or relative weights) of thyroid gland, higher levels of TSH, and lower levels of TT_4 and TT_3 in rats receiving sodium perchlorate (1%) in drinking water for 5 weeks. In agreement with earlier reports [11,13,14] there was no enhancement of LP in any of the tissues examined, but striking AOE alterations were observed in the thyroid gland. Despite no alterations in GSHPx activity in any other tissue, thyroidal GSHPx increased ~2.5-fold compared with control rats. This was in accordance with the results of earlier reports [23–26]. In fact,

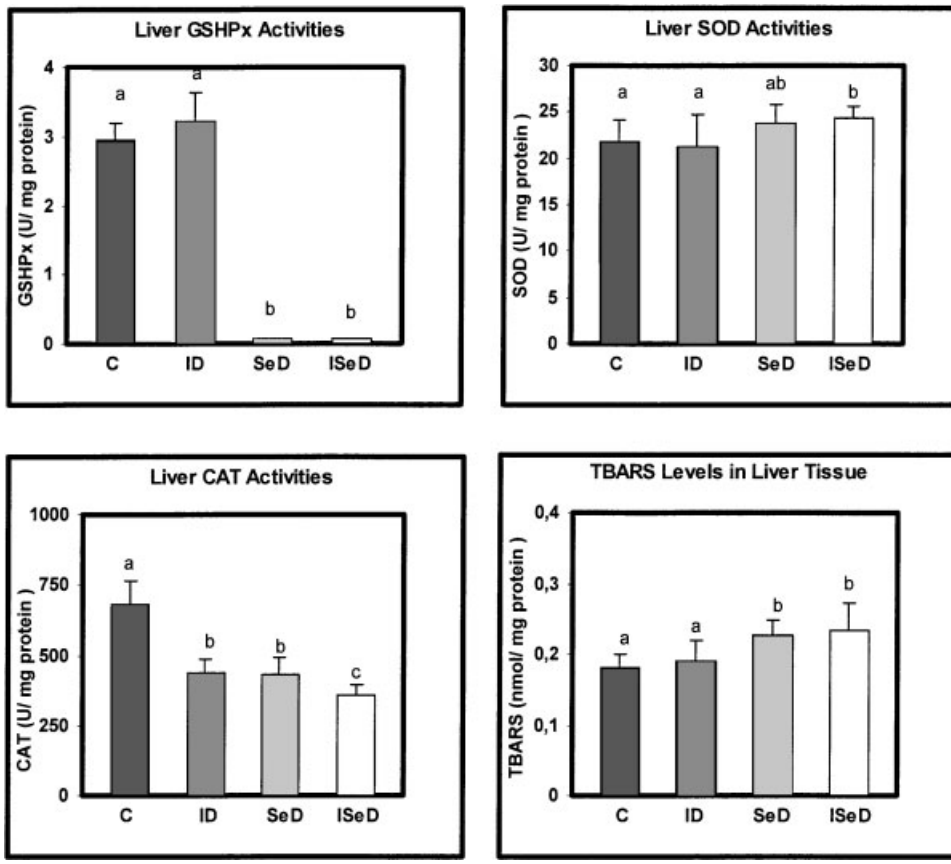

Fig. 2. AOEs and LP levels in liver tissues of selenium- and/or iodine-deficient rats. Superscripts of different letters differ significantly ($P < 0.05$) from each other. For abbreviations, see legend to Fig. 1.

Zagrodzki et al [25] observed a 2- to 4-fold increases in thyroidal GSHPx in iodine-deficient heifers, in addition to a 10- to 12-fold induction of thyroidal type I iodothyronine deiodinase (ID-I). The activities of the two enzymes were not affected in liver, kidney, pituitary, and brain by iodine deficiency. The same authors also reported that a 5-fold increase in the mRNA level of thyroidal GSHPx and an increase in the activity in second generation iodine-deficient rats, suggesting that the iodine deficiency may produce an oxidant stress on the thyroid gland increasing the requirement for selenium to maintain selenoenzyme activity [26].

In the present study, almost 100% increase in thyroidal CAT activity was also observed, but the increase in SOD was not significant. All these alterations, thus, indicated the availability of an adaptive response mechanism in AOE system to protect the thyroid against oxidative stress induced by the excessive generation of H_2O_2 and the reactive oxygen species (ROS) derived thereof. The production of H_2O_2 is essential for the normal control of thyroid hormone synthesis. Selenoenzyme systems in the thyrocytes are thought to be the main components of the

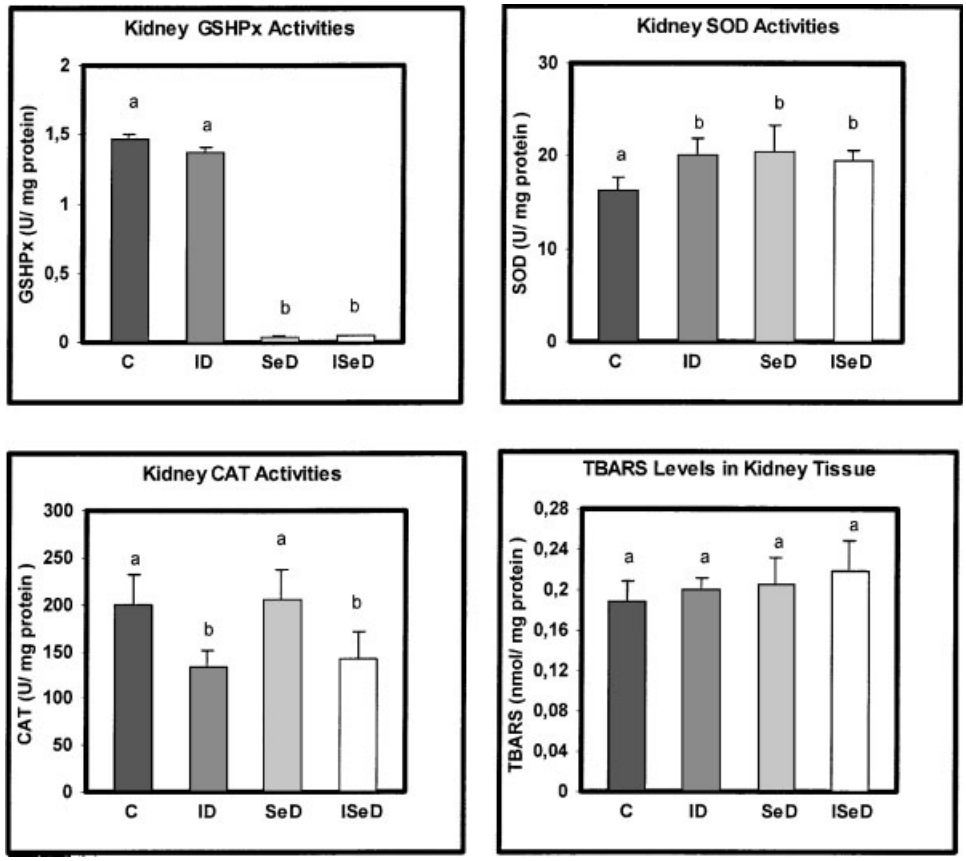

Fig. 3. AOEs and LP levels in kidney tissues of selenium- and/or iodine-deficient rats. Superscripts of different letters differ significantly ($P < 0.05$) from each other. For abbreviations, see legend to Fig. 1.

cell defense regulating the availability of H_2O_2 and subsequent peroxidative damage, as well as the thyroid hormone synthesis. Thyrocytes seem to be protected by the activities of SOD and CAT, as well. In case of iodine deficiency, they are stimulated by higher levels of TSH to produce excessive amount of H_2O_2. Consequently, the gland may be exposed to greater amounts of H_2O_2, which may be the precursor of highly reactive peroxides. However, our results in ID rats indicated that the elevated activities of GSHPx and CAT in thyroid provide effective means of elimination and control of H_2O_2 and thus prevent the generation of LP.

Thyroid has a higher selenium retention factor. Selenium concentration is higher in the gland than in any other tissue other than liver and kidney, indicating that selenium has important functions within the thyroid [27]. It is apparent that GSHPx activity is differentially regulated in the thyroid compared with other tissues. In fact, as was reported by Hotz et al. [23] and Zagrodzki et al. [25], thyroidal GSHPx of ID rats was markedly higher in the present study. The higher activity of thyroidal GSHPx in response to iodine deficiency appeared to

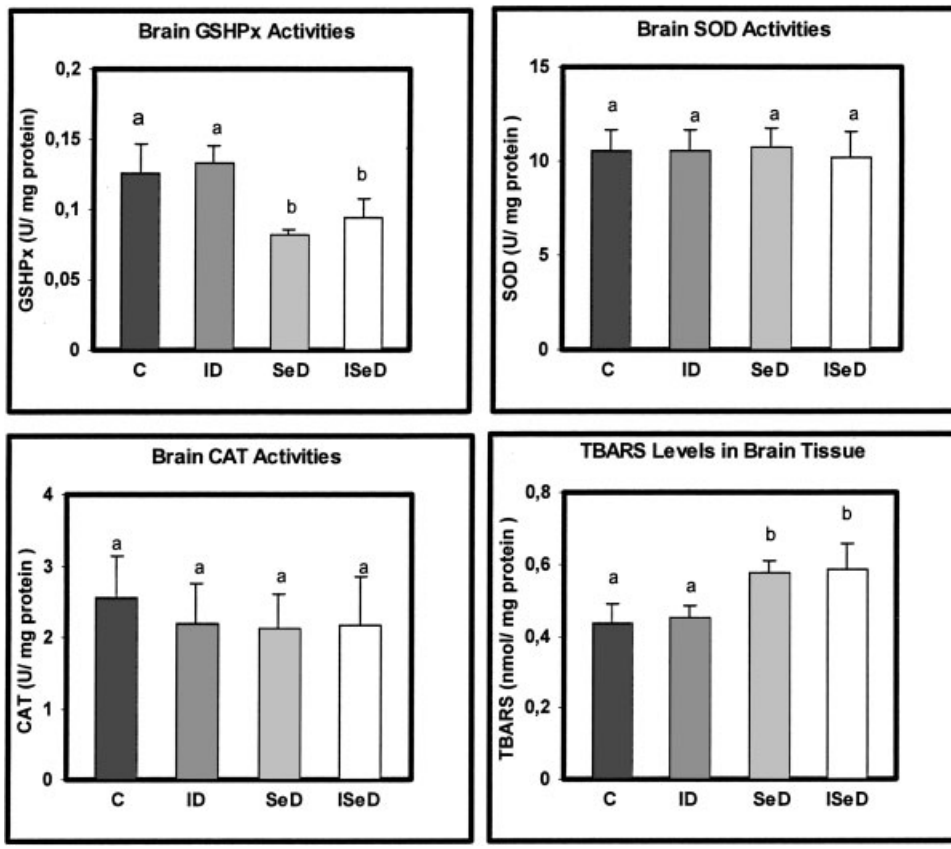

Fig. 4. Antioxidant enzyme activities and lipid peroxidation levels in brain tissues of selenium and/or iodine deficient rats. Superscripts of different letters differ significantly ($P < 0.05$) from each other.

be specific to the thyroid, since in other tissues examined it remained to be unaffected.

Furthermore, as pointed out by Mitchell et al. [26], the level of iodine availability has a greater effect on the activity of thyroidal GSHPx than did the level of available selenium, and this is also evident with our results both in iodine deficiency alone, or combined selenium and iodine deficiency. Brain seemed to be well protected, and the AOEs of RBC and plasma LP were also unaffected.

The only alterations observed in other tissues in ID rats were higher SOD activity in kidney and lower CAT activity in kidney and liver. The reduction of CAT activity in liver was in agreement with the study of Das and Chainy [13], overall results of which suggested that the mitochondrial antioxidant defense system in rat liver, including the glutathione metabolism is considerably influenced by the thyroid states of the body. Thyroid hormones increase the metabolic activity of almost all tissues of the body and known to influence several mitochondrial functions including oxygen consumption, oxidative phosphorylation and proton leak [16,28,29]. Therefore, variations of thyroid hormone levels can be one of the main physiological modulators of in vivo cellular oxi-

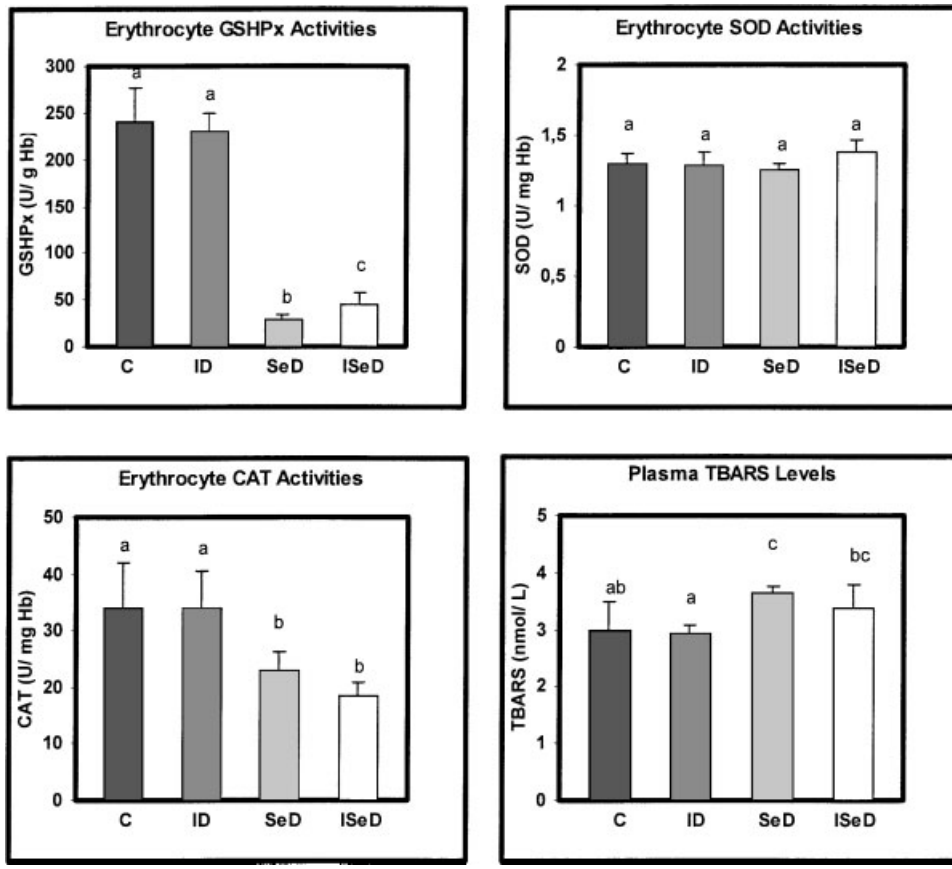

Fig. 5. Erythrocyte AOES and plasma LP levels in selenium- and/or iodine-deficient rats. Superscripts of different letters differ significantly ($P < 0.05$) from each other. For abbreviations, see legend to Fig. 1.

dative stress. Hypermetabolic state in hyperthyroidism is associated with increases in free radical production and LP levels, and the hypometabolic state in hypothyroidism is generally associated with a decrease in free radical production and LP products [11,14,30]. Administration of T_3 to hypothyroid rats was associated with increased oxidative capacity and LP, susceptibility to oxidative stress, and decreased antioxidant levels [11–13]. In hyperthyroidic patients, an increase of LP and AOE and reduction of total antioxidant capacity were reported [31]. However, Rahaman et al. [32] reported that thyroid hormone deficiency in the developing rat brain is accompanied by increased oxidative stress along with an aberrant accumulation of neurofilaments in the hypothyroid neurons. In the study of Venditti et al. [11], vitamin E level and total antioxidant capacity were found to be significantly decreased in hypothyroid rat liver and heart, however, they concluded that the decrease of oxygen consumption compensated these alterations, resulting in an unmodified LP. The results of Pereira et al. [15] showed that the hormones (thyroid hormones, insulin, glucocorticoids) do control the activation of the AOEs and H_2O_2 production both in vivo and in

vitro in macrophages. At present, however, the only clear conclusion of all these data along with the results of present study is that the delicate balance between the rate of formation of ROS and the rate of breakdown of ROS in various tissues of the body is influenced by the thyroid hormones.

In selenium deficiency, the loss of thyroidal GSHPx activity was ~80%, and the enhancement of thyroidal LP was more than 60%. These results pointed out that the gland was not protected against oxidative stress in selenium deficiency despite the presence of ~50% increase in thyroidal CAT activity. In parallel to very low selenium levels, GSHPx activity decreased significantly also in other tissues and RBC of SeD rats. Significant changes in CAT and/or SOD activities in all tissues, except brain, were also noted. Marked enhancement of LP observed in thyroid, liver, brain, and plasma indicated the elevation of oxidant stress was mainly the result of the substantial losses of GSHPx activity in selenium deficiency. There was no modification, however, on LP in kidney. Within the limits of measured parameters of present study, this might only be explained by increased activity of renal SOD that was found to be induced in all three types of deficiency states examined.

Combined iodine and selenium deficiency resulted in higher TT_4, TT_3, and TSH concentrations than those observed in iodine deficiency, reflecting the effects of both iodine and selenium on thyroid hormone synthesis and metabolism. The induction of oxidant stress and, except thyroid, the alterations of AOE observed in combined deficiency were similar to those seen in selenium deficiency. The same degree of increases in LP observed in thyroid, brain, liver, and plasma, but not in kidney, as was seen in selenium deficiency alone. Substantial GSHPx losses were noted in all of the tissues examined, and the extent of depletion in liver, kidney, and brain was the same as in selenium deficiency, whereas it was lower in RBC and thyroid. The alteration in AOE activities in thyroid, however, showed the same trend as seen in iodine deficiency. GSHPx, CAT and SOD activities were higher than the control values, and the activities of CAT and SOD increased by 150% and 70%, respectively. But, the increase in GSHPx was much lower (~35%) than seen in iodine deficiency. These results again point out that the deficiency of iodine produces oxidant stress on the thyroid gland, increases the requirement for selenium to maintain the selenoprotein defense system, and the level of iodine availability has a greater effect on thyroidal GSHPx activity. According to the results of Mitchell et al. [26], in fact, when dietary supplies of selenium are limiting, thyroid selenoprotein mRNA levels increase to compensate for overall lack of the micronutrient, and there is a preferential supply of available selenium to GSHPx (and to ID-I) to allow maintenance of thyroid function. As evidenced in the present study, however, neither the preferential supply of selenium nor the compensatory augmentation of CAT and SOD activities can protect the thyroid gland against excessive production of ROS in the case of severe combined deficiency.

CONCLUSION

The results of this study demonstrated that the two integral components of the thyroid hormone system, iodine and selenium, affect significantly antioxidant

defense in various tissues of rats. In iodine deficiency there occurs marked AOE alterations in the thyroid gland so that the gland protects itself against oxidant stress generated by excessive TSH induction. But, iodine deficiency does not cause any alterations leading LP on the other tissues. Selenium deficiency or combined iodine and selenium deficiency, however, cause oxidant stress in various tissues including thyroid. This appears to be caused mainly by the depletion of selenium reservoirs and accordingly to the substantial losses of GSHPx, although compensatory changes in SOD and/or CAT activities are also triggered.

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