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DIETARY SELENIUM INTAKE AND ANTIOXIDANT DEFENSES IN TISSUES OF PERIPUBERAL RATS

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1. INTRODUCTION

Free radicals are highly reactive chemical species that can oxidize and damage essential biological molecules. Their formation is a result of endogenous metabolism or of xenobiotics bio-transformation, but under normal physiological conditions cells are protected against oxidative challenge by enzymatic and non enzymatic antioxidants (Sies and Groot, 1992; Yu, 1994). Selenium (Se) is a trace element which essentiality for animals and humans is now well established. The element performs its functions mainly through selenoproteins and several glutathione peroxidases that degrade hydroperoxides using glutathione (GSH) as a reducer, are selenoenzymes playing an important role as antioxidant defenses (Ursini, Maiorino and Gregolin, 1985; Flohé, 1989). However, selenium is also a toxic agent with a narrow range of suitable levels.

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The main objective of this study was to assess the physiological development of some antioxidant parameters in liver and testes of rats during the puberty until the adult hood and to know if a low selenium supplementation in the diet can have some effect on those parameters, especially on those related with glutathione metabolism.

2. ANIMALS AND METHODS

2.1. Animals and Diets

This study was carried out with about eighty male Wistar rats. Twenty one days old animals (weaning rats) were fed a commercial standard diet containing 0.1 ppm Se or this diet supplemented with 0.5 ppm Se as sodium selenite in the drinking water. Five age groups were selected for study: 4, 6, 8, 10 and 16 weeks of age.

2.2. Methods

Plasma selenium concentrations were measured by electrothermal atomic absorption according to Nève, Chamart and Molle (1987).

Se-glutathione peroxidase (Se-GSHPx) was assayed with hydrogen peroxide as substrate according to Lawrence and Burk (1976).

Glutathione transferase (GSH S-Tr) activity was measured using the method of Habig, Pabst and Jakoby (1974) with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

The activity of glutathione reductase (GSSG Rd), the enzyme that regenerates GSH was measured using the method developed by Pinto and Bartley (1969).

Glutathione contents were assessed by the 5-5′-dithiobis-(2-nitrobenzoic acid) (DTNB)—GSSG reductase recycling assay, as described by Anderson (1985), in tissue samples pulverised under liquid nitrogen.

3. RESULTS

Means of plasma selenium levels increased during the puberty in both normal and supplemented rats (Table 1), but the differences between the dietary groups at identical ages were not statistically significant.

As it was expected, in the liver the Se-GSHPx activity increased during puberty and from about 8 weeks of age it stayed at levels concordant with adulthood

Table 1. Plasma selenium levels of normal and supplemented rats during the puberal development until the adult age

	Selenium (μg/L)	
Age (weeks)	Normal groups	Supplemented groups (0.5 ppm Se)
4	461 ± 107	—
6	389 ± 85	—
8	472 ± 99	588 ± 80
10	521 ± 141	661 ± 60
16	595 ± 86	668 ± 90

Results are mean ± SD, based on 4–10 rats per age group.

Table 2. Effect of a low dietary Se supplementation on enzyme activities in liver of rats during the puberal development until the adult age

	Se-GSHPx (μmol NADPH oxid./min/g wet tissue)		GSHS-Tr (μmol product/min/g wet tissue)	
Age (weeks)	Normal groups	Suppl. groups (0.5 ppm Se)	Normal groups	Suppl. groups (0.5 ppm Se)
4	53.5 ± 4.8	—	71.3 ± 11.7	—
6	75.4 ± 11.8	93.2 ± 17.0	100.4 ± 10.0	131.3 ± 15.1*
8	94.4 ± 10.3	106.0 ± 8.2	102.4 ± 8.2	128.9 ± 9.9*
10	86.2 ± 10.7	90.6 ± 19.6	99.2 ± 6.8	127.4 ± 12.0*
16	96.3 ± 13.9	120.0 ± 19.1*	116.8 ± 14.4	141.4 ± 13.8*

Results are mean ± SD, based on 4–8 rats per age group. *$P < 0.05$, based on the t-student test.

(Table 2). When the diet was enriched with Se, a significantly higher activity in liver of young adults (16 weeks of age) was observed. The activity of GSHS-Tr increased in liver until the age of 6 weeks and after that it stayed unchanged (Table 2). The dietary selenium supplementation readily led to higher hepatic GSHS-Tr activity.

In testes, the Se-GSHPx activity also increased during the puberty, but any change with the diet enriched with selenium was not observed (Table 3). On the other hand the activity of GSHS-Tr raised in testes only after maturity (8 weeks of age). Once more, the selenium supplementation in the diet had no effect on this testicular parameter.

GSSG reductase activity and glutathione contents were mesured in the adult age groups. The diet with higher selenium level led to an increased content of total glutathione in liver, but the level of the oxidised form as well as the activity of its reductase were unchanged (Table 4). Any difference in the testicular parameters could not be detected.

4. DISCUSSION

In Wistar rats, the development of Se-GSHPx and GSH S-Tr activities from weaning to the adult age is different in the two tissues. On the other hand, the hepatic activities are always higher than those measured in the testes. In the liver, the findings related to the first enzyme are similar to those observed by others (Pinto and Bartley, 1969), but the GSH S-Tr activity has an evolution which is different from some data in

Table 3. Effect of a low dietary Se supplementation on enzyme activities in testes of rats during the puberal development until the adult age

	Se-GSHPx (μmol NADPH oxid./min/g wet tissue)		GSHS-Tr (μmol product/min/g wet tissue)	
Age (weeks)	Normal groups	Suppl. groups (0.5 ppm Se)	Normal groups	Suppl. groups (0.5 ppm Se)
4	1.7 ± 0.5	—	32.4 ± 6.1	—
6	2.3 ± 0.5	2.4 ± 0.2	32.2 ± 3.2	32.8 ± 3.7
8	3.0 ± 0.6	3.2 ± 0.5	32.6 ± 3.4	33.2 ± 6.6
10	3.3 ± 0.5	3.4 ± 0.8	41.5 ± 7.5	44.8 ± 4.0
16	3.4 ± 1.0	3.9 ± 0.9	47.6 ± 7.2	47.5 ± 5.3

Results are mean ± SD, based on 4–8 rats per age group.

Table 4. Effect of a low dietary Se supplementation on glutathione levels and glutathione reductase activity in liver and testes of rats at the age of sixteen weeks

	Liver		Testes	
Parameter	Normal group	Suppl. group	Normal group	Suppl. group
Total GSH (nmol/g wet tissue)	7,225 ± 897	9,116 ± 568*	3,474 ± 487	3,933 ± 397
GSSG (nmol/g wet tissue)	100 ± 16	90 ± 19	25 ± 11	23 ± 6
GSSG Rd (U*/g wet tissue)	8.7 ± 0.6	7.8 ± 1.0	0.8 ± 0.1	0.8 ± 0.1

*U is defined as nmol of GSSG reduced/min. Results are mean ± SD, based on 5–7 rats per age group. *$P < 0.05$, based on the t-student test.

the literature (Peltola, Huhtaniemi and Ahotupa, 1992; Jung and Henke, 1996). When animals are fed the selenium supplemented diet, the liver shows to be sensitive to small changes of dietary selenium contents as it has been found elsewhere (Crespo, Pinto and Neve, 1993).

In the testes, the developmental profile of Se-GSHPx is also according to that observed by Behne, Duk and Elger (1986) and it is very different from the GSH S-Tr profile. As it was expected, in these conditions the testes are not affected by selenium supplementation, due to efficient testicular homeostatic mechanisms (Behne, 1989).

This study suggests that selenium in diet at moderated levels, that are not much higher than the recommended intake of the element for these animals, has influence on some steps of hepatic glutathione metabolism, increasing the activity of some enzymes related to GSH and also the level of this thiol, probably as a result of an adaptive response.

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