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EFFECT OF STARVATION AND IMMOBILIZATION ON GLUTATHIONE LEVEL AND ACTIVITY OF GLUTATHIONE ENZYMES IN THE LIVER AND KIDNEY OF RABBITS

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ABSTRACT

The study was carried out on 6-months-old male and female New Zealand rabbits. The animals were divided into 3 groups: control, 72 hours starved and 72 hours immobilized.
The level of the GSH and activity of glutathione enzymes decreased after starvation and immobilization in the liver and kidney of experimental animals.

Key words: glutathione, enzymes, rabbits, starvation, immobilization

Immobilization and starvation are one of the most powerfully operated stressors. Animals deprived of freedom generate very strong defense reactions, mainly neurogenic, which originates from the central nervous system. During the first stress period of immobilization they are not interested in food or in drinking. In the state of hunger they consolidate new homeostatic balance and can utilize proteins from their own body as a source of energy (Balutsov,1989; Bhardwaj et al., 1998; Bray and Taylor, 1993; Cai, 2003; Domenicali et al., 2001; Grattagliano et al., 2000; Kołątaj et al., 1995; Kvetnansky, 1993; Mileve et al., 2002; Oishi and Machida, 2002).

Glutathione (GSH) is an important water-phase antioxidant and essential cofactor for antioxidant enzymes. Glutathione concentration is often considered an indicator of cell vitality (Bray and Taylor, 1993; Cai, 2003; Cho et al., 1981; Jocelyn, 1972; Meister, 1994).

The glutahione S-transferases [EC 2.5.1.18] are a complex family of multifunctional enzymes involved in the detoxification of wide spectrum of compounds, such as environmental pollutants, carcinogens and mutagens, as well as endogenous toxic compounds (Jakoby and Habig, 1980; Prabhu et al., 2001.

Glutathione peroxidase [EC 1.11.1.9] is an intracellular enzyme and it belongs to several proteins in mammalian cells that can metabolize hydrogen peroxide and lipid hydroperoxides (Potačkova et al., 2003). Since glutathione plays an important role in regulation of metabolic processes it seemed interesting to determine its concentration, activity glutathione peroxidase and activity glutathione transferase in the liver and kidney of the rabbits during the starvation and immobilization applied as the model stressors yet existing in the animals breeding.

MATERIAL AND METHODS

The study was carried out on 60 New Zealand male and female rabbits of 3 months of age, weighed 2.5-3 kg and were divided randomly into control and experimental groups in the farm of the Department of Animal Genetics in the Agricultural University in Cracow. All animals were fed standard industrial granulated feed for rabbits (16% of protein) and carrots. The access to water was available in their cages. In the farm the ventilated hall had a temperature regime of 18° -22°C and 50:50 dark/light. The immobilized rabbits also were fed. All animals received good veterinary care. First group of experimental rabbits were starved for 72 hours and the second group was immobilized for 72 hours. Water was supplied *ad libitum* to all the animals.

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The control and starved individuals remained in their normal cages (80 cm x 60 cm x 50 cm). The immobilized animals were kept in the special small cages (40 x 20 x 20 cm) which were placed onto their normal cages. They could eat their food, drink water, placed within reach of the head movements.

After the experiments all the animals were killed by breaking of spinal cord, always between 9^{oo} and 11^{oo} a.m. The livers and kidneys were immediately isolated. Liver was subjected to perfusion by a solution of physiological salt cooled to 4^{o}C. The weighed 600 mg slices were then homogenized in 6 ml 0.1 M phosphate buffer (pH 7.4) with 10 mM EDTA, in a potter type homogenizer with a teflon piston at 200 rot./min. Homogenates were centrifuged for 15 min. at 12 000 rot/min. in a Janetzky centrifuge K-24. In the supernatants the content of glutathione in mmol/g of tissue was determined by the Ellman´s (1959) method, glutathione transferase activity [U/mg of protein/min.] according to Habig et al. (1974), glutathione peroxidase by Chiu et al. (1976) method and total protein according to Lowry et al (1951) method modified by Kirschke and Wiederanders (1984). The enzymatic substrates used were purchased from Sigma.

Statistical analyses were carried out with ANOVA, Fisher´s test, and t-test. Differences were accepted as significant at the level $p< 0.05$.

RESULTS AND DISCUSSION

Table 1 shows a statisticant confirmed decrease in GSH concentration in the liver after starvation to 65% in females and 49% in males, and in the kidney to 70% and 78% respectively. After immobilization a decrease in GSH was found in the liver (to 76% and 63% respectively), whilst in the kidney no change was noted (to 97% and 92% respectively). Starvation and immobilization induced changes were greater in males than in females and the liver reacted more intensively to these stimuli than the kidney (Borras et al., 2003).

In the control rabbits the glutathione level was higher in the liver than in the kidney and the control males had higher values of GSH in both the organs than the control females. Thus, our studies confirmed the data obtained by other authors (Creighton, 1983; Jocelyn, 1972; Świderska-Kołacz et al., 2001; Świderska-Kołacz et al., 1997) that glutathione content is higher in the rabbit liver than in the kidney. Some authors reported that GSH level decreased rapidly in animals fed either with non-protein diets and/or starved (Bhardwaj at al., 1998; Brooks et al., 1981; Cho El Soon et al., 1981), as well as exposed to different kinds of stresses (Kołątaj, 1993; Świderska-Kołacz and Kołątaj, 1994; Świderska-Kołacz et al., 2001; Świderska-Kołacz et al., 1997). Lauterburg

Table 1: Changes in the level of reduced glutathione and activity of glutathione enzymes (`x±SD and %) in the liver and kidney of experimental rabbits after starvation and immobilization; in each subgroup n = 15

GSH and enzymes	Organ	Control		Starvation		Immobilization	
		Females	Males	Females	Males	Females	Males
Glutathione (mM/g)	Liver	9.87 ±1.03	10.90 ±1.41	6.41** ±0.94	5.39*** ±0.83	7.47* ±1.13	6.69** ±1.01
	% from control	100	100	64.94	49.49	75.68	61.38
	Kidney	3.03 ±0.75	3.46 ±0.63	2.12** ±0.42	2.71* ±0.44	2.94 ±0.37	3.20 ±0.33
	% from control	100	100	69.97	78.32	97.02	92.63
Glutathione peroxidase (U/mg)	Liver	0.149 ±0.025	0.132 ±0.026	0.122* ±0.017	0.101* ±0.021	0.119* ±0.023	0.111* ±0.012
	% from control	100	100	81.88	76.51	79.86	84.09
	Kidney	0.0532 ±0.0117	0.0600 ±0.014	0.0395* ±0.0055	0.0518* ±0.093	0.0459* ±0.0096	0.0549 ±0.0093
	% from control	100	100	74.25	86.19	86.27	91.35
Glutathione transferase (U/mg)	Liver	8.27 ±1.07	9.12 ±1.73	5.64** ±0.90	6.67* ±1.40	6.06* ±1.82	7.67* ±1.15
	% from control	100	100	68.20	73.13	73.28	84.10
	Kidney	2.43 ±0.41	3.30 ±0.67	1.85* ±032	2.19 ±0.55	1.97* ±0.43	2.44* ±0.51
	% from control	100	100	76.13	88.18	81.07	73.94

*differences statistically significant; *p<0.05; **p<0.01; ***p<0.001

Table 2: Body weight [$\bar{x}$ ±SD (g) and percent] of rabbits after 72h of starvation and 72h of immobilization; in each subgroup n = 30

	Control		Starvation		Immobilization	
Group	$\bar{x}$ ±SD	%	$\bar{x}$ ±SD	%	$\bar{x}$ ±SD	%
Males	2830 ± 305	100	2422 ± 285	85.60	2485* ± 312	87.80
Females	2690 ± 242	100	2194 ± 215	81.60	2319 ± 343	86.20

*differences statistically significant; *p<0.05

and Mitchell (1981) starved experimental rats over 48 hours revealed a marked decrease in glutathione content in their livers. Concentration of GSH, however, returned to normal control values after the resumption of nutrition. Similar data were reported by Cho et al.(1981).

It is known that immobilization evokes the limitation of movement activity and other adjustment capabilities, including the disturbances in metabolic balance too (Liu et al., 1994; Liu et al., 1996; Madrigal et al., 2001; Mileve et al., 2002; Oishi and Machida, 2002; Zorkina et al., 1997). We have noticed during the immobilization a statistically significant decrease in SH levels too, the liver reacting deeper than the kidney.

It is known that the normal physiological concentration of glutathione in the liver cells keeps the balance the glutathione enzymes Deneke and Fanburg, 1989Meister, 1994). Starvation and immobilization decreased the activity of glutathione peroxidase in the liver of both sexes and in the kidney after starvation. The activity of glutathione transferase decreased significantly in all thr organs of males and females.

From obtained results, it may be concluded that during stress burden the sulfhydryl groups of an organism can be mobilized for the defense of metabolism. This refers to an increased requirement of glutathione in the cells. It is possible that in these conditions oxidative stress develops in the tissues too (Bhardwaj et al., 1998; Liu et al., 1996; Madrigal et al., 2001).

Table 2 shows a decrease in rabbit body weight after starvation and immobilization. Similar data were also obtained by Faber at al. (2002).

We suppose that GSH concentration and activity of glutathione enzymes in studied organs can be markers of reactions after stress.

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