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Effect of reduced glutathione (GSH) on activity of lysosomal system in subcellular fractions of mouse kidney

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Investigated was the effect of intraperitoneal injections (twice daily for seven consecutive days) of reduced glutathione (GSH) on activity of selected lysosomal hydrolases (alanine aminopeptidase, leucine aminopeptidase, cathepsins D and L, acid phosphatase and N-acetyl-β-D-glucosaminidase) in lysosomal, microsomal and cytosol fractions of mouse kidney. Most notable effect of exogenous GSH on the activity of the enzymes considered was observed in lysosomal, while the least in microsomal fraction. After a series of GSH injections the activity of enzymes increased significantly mainly in lysosomal fraction. It is assumed that the increase in activity of lysosomal enzymes following the GSH injections results from a physiological response of renal cells to this antioxidant.

KEY WORDS: glutathione / hydrolases / kidney / lysosome / mouse

The kidney is a heterogeneous tissue containing numerous cell populations, each possessing distinct morphological, physiological and biochemical properties. Distal tubular regions of the nephron are considerably more sensitive to oxidative injury by exogenous peroxides or thiol-alkylating agents than are proximal tubular regions [Lash *et al.* 1998]. There are several defense mechanisms in a kidney to minimize oxidative stress [Kowluru *et al.* 1997].

Reduced glutathione (γ-glutamyl-cysteinyl-glycine – GSH) is an antioxidant playing an important role in a redox potential regulation, detoxication of xenobiotics and free radicals, and also serving as a store and transport form of cysteine [Sies 1999].

A regulation of cellular GSH status in the kidney as well as in other tissues is compartmentalized, involving the processes occurring in the plasma membrane, cytosol, endoplasmic reticulum, nucleus, and mitochondria [Lash *et al.* 1998]. Differences in the ability of the cells to use GSH for detoxication and maintenance of redox homeostasis can determine their susceptibility to oxidative injury. In the cells there are two sites where oxidative-damaged molecules are degraded: mitochondries and lysosomes, the intracellular hydrolytic degradation taking place mainly in the latter [Dean *et al.* 1997, Brown *et al.* 2000]. Lysosomes seem especially sensitive to oxidative stress [Hellquist *et al.* 1997, Kołątaj *et al*. 2001, Śliwa-Jóźwik *et al.* 2002]. The present paper aims at analysing changes in the activity of selected lysosomal hydrolases in lysosomal, microsomal and cytosol fractions of mouse kidney as affected by a series of intraperitoneal injections of GSH.

Material and methods

Animals

Used were Swiss mouse males, weighing 25±12 g, maintained under standard conditions in an air-conditioned cubicle at 21°C with a natural photoperiod. The animals were fed a standard commercial diet (16% protein), and had free access to water. Two groups of mice were used – experimental and control (10 animals in each). Reduced glutathione GSH (γ-Glu-Cys-Gly, SIGMA-ALDRICH CHEMIE Gmbh, Germany) was intraperitoneally injected to mice of experimental group at 8 a.m. and 4 p.m. for seven consecutive days, leading to a total of 14 injections per mouse. A single GSH dose was 100 μg/g body weight. To reach this, GSH solutions in 0.9% NaCl were prepared and injected each time in a single dose of 12 μL/g body weight. The mice of the control group received intraperitoneally 12 μL 0.9% NaCl/g body weight each time. Twelve hours after the last injection all animals were killed by decapitation and their kidneys were immediately removed.

The experiment was approved by the Institute's Commission for Ethics in Animal Research.

Preparation of cellular fractions

All procedures were conducted at 0-4°C. Both kidneys were rinsed with 0.9% NaCl, then suspended in a buffer containing 0.25 M saccharose and 2mM EDTA with a ratio of 1 g tissue to 7 mL buffer, and homogenized in a Potter-Elvehjem-type homogenizer at 200 rpm. This procedure does not labilize lysosomes. The preparations of subcellular fractions were obtained according to Marrzella and Glaumann [1980]. The complete homogenates were centrifuged for 10 min in centrifuge K-24, at 700 × g, to remove nuclei and partially broken cells. The supernatant was centrifuged for 20 min at 20,000 × g with the SORVALL RC5C centrifuge. The resulting sediment pellet was considered the lysosomal fraction (L). The supernatant was centrifuged again at 150,000 × g for 60 min with the BECKMAN L7-65 vaccum ultracentrifuge. The resulting sediment

was considered microsomal (M), while the supernatant – cytosol (C) fraction. L and M pellets were suspensed in 0.1 M phosphate buffer containing 0.1% Triton X-100 (pH 6.0). Obtained fractions were frozen-thawed three times. Next, L and M fractions were centrifuged for 5 minutes at 700 × g with K-24 centrifuge.

Biochemical assays

The activities of soluble lysosomal hydrolases were determined in fractions L, M and C. The activity of alanine aminopeptidase (AlaAP - E.C. 3.4.11.2) and leucine aminopeptidase (LeuAP – E.C. 3.4.11.1) were assayed according to McDonald and Barrett [1986] with L-alanine β-naphtylamide and L-Leucine β-naphtylamide as substrates, respectively, at 540 nm. Activity of cathepsin D (E.C. 3.4.23.5.) and cathepsin L (E.C. 3.4.22.15.) were determined according to Langner *et al*. [1973] with azocasein as a substrate, at 366 nm. Acid phosphatase (AcP – E.C. 3.1.3.2) and N-acetylo-β-D-glucosaminidase (NAG – E.C. 3.2.1.30) were assayed after Barrett and Heath [1972] with p-Nitrophenyl phosphate-disodium salt and p-Nitrophenyl-N-acetyl-β-D-glucosaminide as substrates, respectively, at 420 nm. Total enzyme activities were measured after incubation at 37°C and were expressed in nmol/mg of protein/hour. Total protein content was measured with the Lowry's method, modified by Kirschke and Wiederanders [1984] with bovine serum albumin as a standard. All determinations were performed with Lambda Bio 20-1998 spectrophotometer (PERKIN ELMER).

Statistical

The results are presented in Figures as means and their standard deviations (SD) for activity of each enzyme in each fraction in comparison with control (0.9% NaCl) group. The data were analysed by two-way analysis of variance, using the Kramer's test [SAS, 1999-2001].

Results and discussion

It has been shown by Sen [1997] and Regan and Guo [1999] that administered GSH is not effectively transported into the cells except a small intestine. GSH is mostly degraded extracellularly by γ-glutamyl transpeptidase (γ-GT) into glutamate and cysteinyloglycyne, which are both rapidly transported into the cells. Then GSH is resynthesized from its constituent amino acids by the ezymes of γ-glutamyl cycle. The kidneys show high γ-GT activity and are the main organs in which the γ-glutamyl cycle appears in mammals [Griffith 1999]. Our observations show that GSH affected mostly the activity of enzymes in L fraction, whereas the least effect was observed in M fraction what presumably can be attributed to a subcellular distribution of glutathione. Lu [1999] revealed that the eukaryotic cells have three major reservoirs of GSH. Almost all cellular GSH is present in the cytosol and in mitochondria (about 90 and 10%, respectively), and a meaningless per cent is found in the endoplasmic reticulum (ER). Within the ER a GSH:oxidized glutathione ratio is 2:1, whereas a cytosolic ra-

tio ranges from 30:1 to 100:1 [Banhegyi *et al*. 1999, Csala *et al*. 2000]. The oxidized environment within the ER, independently on cytosolic GSH contents, is necessary for the formation of disulfide bonds and for the proper folding of proteins transported along the secretory pathway [Lu 1999, Banhegyi *et al.* 1999]. Lysosomal proteins are synthesized as precursors, which are posttranslationally processed to the mature lysosomal forms, during their passing through the Golgi apparatus. It may be connected with a smaller enzymes activity found in a microsomal fraction. Our study showed that the increase in the estimated enzymes' activity after a GSH injections took place mainly in L fraction. The lysosomal enzymes are widely distributed in the kidney [Rudiger *et al*. 1998, Iwata *et al*. 1999, Jin *et al.* 1999]. They are located predominantly in the brush border membrane (AlaAP, LeuAP) and the glomerular basement membrane of proximal tubular (PT) and distal tubular (DT) regions of the nephron (NAG, AcP). Lash *et al.* [1998] showed that GSH is transported out of renal PT cells across the brush border membrane by a membrane potential-sensitive carrier. Our study suggests that GSH can activate the filtration barrier and stimulate a secretory activity of the tubular cells through increasing lysosomal enzyme activities. It is known, that GSH injection leads to increased GSH content of blood and some organs in mice [Witek and Kołątaj 1998, Śliwa-Jóźwik *et al*. 2002].

This study shows that in the mouse kidney the highest aminopeptidase activity is that of lysosomal fraction. In both groups of mice activities of AlaAP (Fig. 1) and LeuAP (Fig. 2) were found to be highest in L fraction.The GSH injection led to a significant increase in aminopeptidases activity of the L fraction and a slight decrease in that of

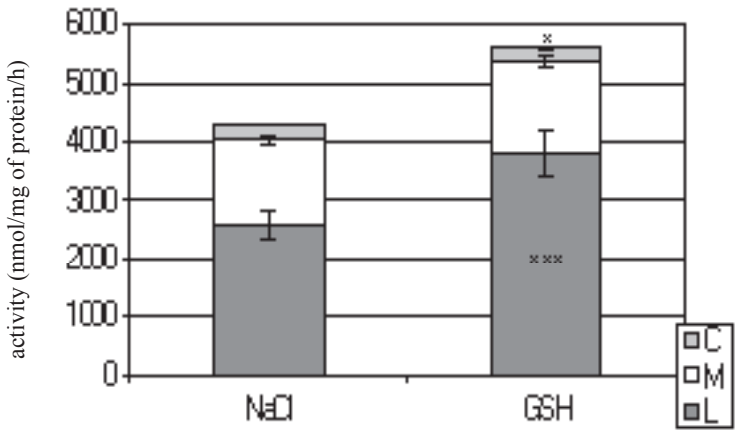

Fig. 1. Changes of AlaAP activity in lysosomal, microsomal and cytosol fraction after GSH injection. The data are presented as means ± SD.
*,***Differences between group means are significant at $P<0.05$ and $P<0.001$, respectively.

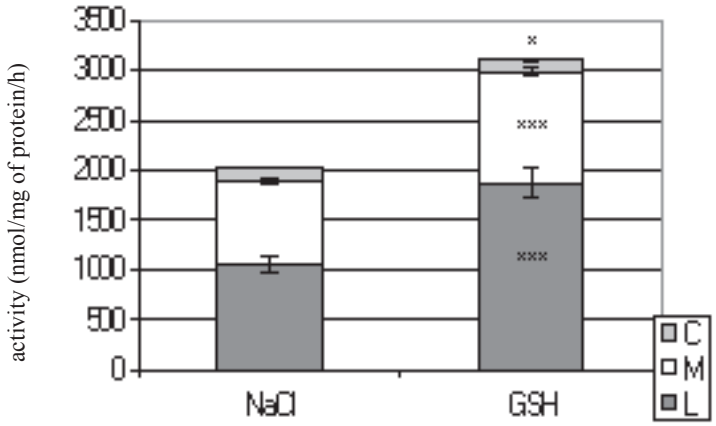

Fig. 2. Changes of LeuAP activity in lysosomal, microsomal and cytosol fraction after GSH injection. The data are presented as means ± SD.
*,***Differences between group means are significant at P<0.05 and P<0.001, respectively.

fraction C (P≤0.001 and ≤0.05, respectively). Chou *et al*. [1998] showed that high level of cysteine derived from extracellular GSH hydrolysis caused an increase in sulphate and taurine content of the lysosomal transport sysytem. We suggest that it can cause an increased lysosomal proteolysis in the brush border membrane of the nephron, including that by AlaAP and LeuAP. The decrease in these two aminopeptidases cytosolic activity suggests that GSH may stabilize the lysosomal membranes and protect the kidney cells from uncontrolled autolysis. This mechanism may be related to the activity of D and L cathepsin, as well as to AlaAP, and LeuAP activities.

As presented in Figure 3, cathepsins D and L (pooled) showed the highest activity in L, and lower in M and C fractions. This confirms the lysosomal localization of cathepsin D and L in the kidney [Chauhan *et al*. 1998] suggesting a stimulatory effect of thiols on intralysosomal proteolysis.

High AcP activity (Fig. 4) was shown in L and C and the least in M fraction, confirming the earlier results of Chen and Chen [1988]. AcP contains two iron atoms existing in a two redox states [Beck *et al*. 1999]. The reduced form Fe(II)-Fe(III) is catalytically active and is readily converted into the inactive Fe(III)-Fe(III) form. The sensitivity of AcP to the changes in the redox potential of a cell may explain an increase in the activity of AcP observed in this study. GSH increased the activity of AcP in L and C fraction (Fig. 4) and it may suggest a protective role of thiols on the activity of AcP of both fractions. We anticipate that it may activate the filtration barrier because AcP in the glomerular basement membrane specifically serves in the clearance of macromolecular debris to facilitate ultrafiltration [Rudiger *et al*. 1998].

The highest activity of NAG was shown in L fraction (Fig. 5), corroborating the

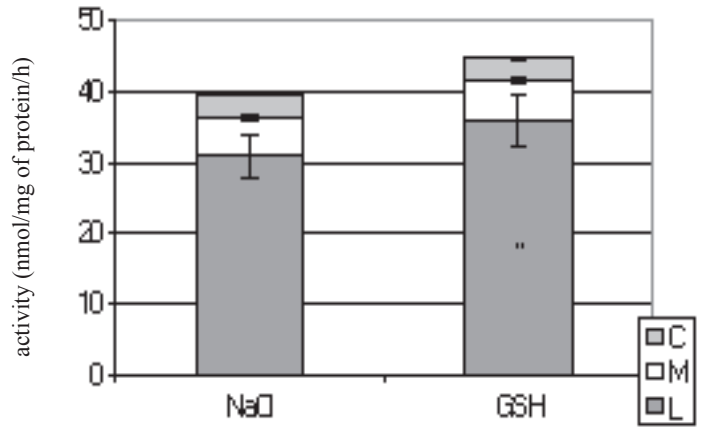

Fig. 3. Changes of cathepsin D and L activity in lysosomal, microsomal and cytosol fraction after GSH injection. The data are presented as means ± SD.
*Differences between group means are significant at P<0.05.

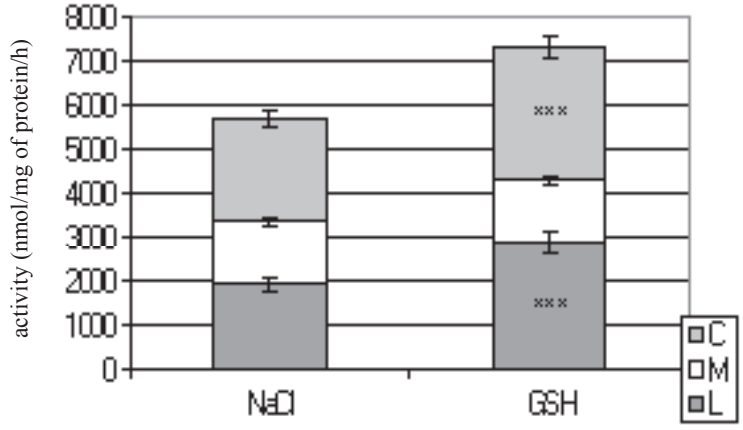

Fig. 4. Changes of AcP activity in lysosomal, microsomal and cytosol fraction after GSH injection. The data are presented as means ± SD.
***Differences between group means are significant at P<0.001.

earlier reports by Iwata *et al*. [1999] and Jin *et al*. [1999]. The former authors suggested that NAG in the kidney is located predominantly in the brush border of proximal tubules where amino acids and vitamins are resorbed. In the present study, injected GSH increased the activity of NAG mainly in C fraction. We suggest that the high GSH concentration results in a higher N-acetylocysteine level and can be associated with the

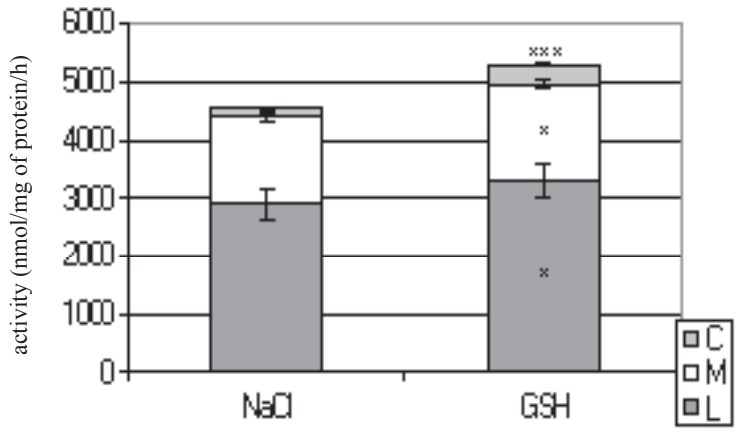

Fig. 5. Changes of NAG activity in lysosomal, microsomal and cytosol fraction after GSH injection. The data are presented as means ± SD.
*,***Differences between group means are significant at P<0.05 and P<0.001, respectively.

damage of lysosomal membranes allowing the enzyme to penetrate into the cytosol. It was shown [Lash *et al*. 1999, Lu 1999] that N-acetylation of cysteinyl amino group results in formation of the N-acetylcysteine that it is easily excreted in the urine.

Concluding, it can be anticipated that exogenous GSH increases the activity of some lysosomal enzymes as a physiological response of renal cells to this antioxidant. The rate of such increase depends on subcellular lysosomal, microsomal or cytosol fraction.

REFERENCES

1. BANHEGYI G., LUSINI L., PUSKAS F., ROSSI R., FULCERI R., BRAUN L., MILE V., SIMPLICIO P., MANDL J., BENEDETTI A., 1999 – Preferential transport of glutathione *versus* glutathione disulfide in rat liver microsomal vesicles. ***Journal of Biological Chemistry*** 274, 12213-12216.
2. BARRETT A.J., HEATH M.F., 1972 – Lysosomal enzymes. In:. Lysosomes. A Laboratory Handbook (J.T. Dingle ed.) North-Holland, Amsterdam, 19-135.
3. BECK J.L., DURACK M.C.A., HAMILTON S.E., DEJERSEY J., 1999 – Irreversible inactivation of purple acid phosphatase by hydrogen peroxide and ascorbate. ***Journal of Inorganic Biochemistry*** 73, 245-252.
4. BROWN A.J., MANDER E.L., GELISSEN I.C., KRITHARIDES L., DEAN R.T., JESSUP W., 2000 – Cholesterol and oxysterol metabolism and subcellular distribution in macrophage foam cells; accumulation of oxidized esters in lysosomes. ***Journal of Lipid Research*** 41, 226-236.
5. CHAUHAN S.S., RAY D., KANE S.E., WILINGHAM M.C., GOTTESMAN M.M., 1998 – Involvement of carboxy-terminal amino acids in secretion of human lysosomal protease cathepsin L. ***Biochemistry*** 37, 8584-8594.

6. CHEN Ch.H., CHEN S.C., 1988 – Evidence of acid phosphatase in the cytoplasm as a distinct entity. ***Archives of Biochemistry and Biophysics*** 262, 427-438.
7. CHOU H.F., PASSAGE M., JONAS A.J., 1998 – Lysosomal sulphate transport is dependent upon sulphydryl groups. ***Biochemical Journal*** 330, 713-717.
8. CSALA M., MILE V., BENEDETTI A., MANDL J., BANHEGYI G., 2000 – Ascorbate oxidation is a prerequisite for its transport into rat liver microsomal vesicles. ***Biochemical Journal*** 349, 413-415.
9. DEAN R.T., FU S., STOCKER R., DAVIES M.J., 1997 – Biochemistry and pathology of radical-mediated protein oxidation. ***Biochemical Journal*** 324, 1-18.
10. GRIFFITH O.W., 1999 – Biologic and pharmacologic regulation of mammalian glutathione synthesis. ***Free Radical Biology & Medicine*** 27, 922-935.
11. HELLQUIST H.B., SVENSSON I., BRUNK U.T., 1997 – Oxidant-induced apoptosis, a consequence of lethal lysosomal leak. ***Redox Report*** 3, 65-70.
12. IWATA T., UCHIDA S., HORI M., SAKAI K., TOWATARI T., KIDO H., 1999 – Human parathyroid hormone (1-34) increases urinary excretion of lysosomal enzymes in rats. ***Life Sciences*** 65, 1725-1732.
13. JIN T., NORDBERG G., WU X., YE T., KONG Q., WANG Z., ZHUANG F., CAI S., 1999 – Urinary N-acetyl-β-D-glucosaminidase isoenzymes as biomarker of renal dysfunction caused by cadmium in a general population. ***Environmental Research*** 81, 167-173.
14. KIRSCHKE H., WIEDERANDERS B., 1984 – Methoden zur Aktivitätsbestimmung von Proteinases. Wissenschaftlichen Beitrage. Martin Luther Universität Halle-Saale, 43-56.
15. KOŁĄTAJ A., ŚLIWA-JÓŹWIK A., JÓŹWIK A., 2001 – The lysosomal cell complex as a stress response indicator. ***Animal Science Papers and Reports*** 19, 177-192.
16. KOWLURU R.A., KERN T.S., ENGERMAN R.L., 1997 – Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. ***Free Radical Biology & Medicine*** 22, 587-592.
17. LANGNER J., WAKIL A., ZIMMERMANN M., ANSORGE S., BOHLEY P., KIRSCHKE H., WIEDERANDERS B., 1973 – Aktivitätsbestimmung proteolytischer Enzyme mit Azokasein als Substrat. ***Acta Biologica et Medica Germanica*** 31, 1-18.
18. LASH L.H., LIPSCOMB J.C., PUTT D.A., PARKER J.C., 1999 – Glutathione conjugation of trichloroethylene in human liver and kidney, kinetics and individual variation. ***Drug Metabolism and Disposition*** 27, 351-359.
19. LASH L.H., VISARIUS T.M., SALL J.M., QIAN W., TOKARZ J.J., 1998 – Cellular and subcellular heterogeneity of glutathione metabolism and transport in rat kidney cells. ***Toxicology*** 130, 1-15.
20. LU S.C., 1999 – Regulation of hepatic glutathione synthesis, current concepts and controversies. ***FASEB Journal*** 13, 1169-1183.
21. MARZELLA L., GLAUMANN H., 1980 - Increased degradation in rat liver induced by Vinblastine. I Biochemical characterization. ***Laboratory Investigation*** 42, 8-17.
22. MCDONALD J.K., BARRETT A.J., 1986 – Exopeptidases. In: Mammalian Proteases. A Glossary and Bibliography 2, London, 111-144.
23. REGAN R.F., GUO Y.P., 1999 – Potentiation of excitotoxic injury by high concentrations of extracellular reduced glutathione. ***Neuroscience*** 91, 463-470.
24. RUDIGER J., KALICHARAN D., HALBHUBER K.J., WANT J.J.L., 1998 – Extralysosomal localisation of acid phosphatase in the rat kidney. ***Histochemistry and Cell Biology*** 109, 375-382.
25. SAS, SAS/ STAT 1999-2001 – User's Guide Release 8.E. SAS Institute Inc., NC, USA.
26. SEN Ch., 1997 – Nutritional biochemistry of cellular glutathione. ***Journal of Nutritional Biochemistry*** 8, 660-672.

27. SIES H., 1999 – Glutathione and its role in cellular functions. ***Free Radical Biology & Medicine*** 27, 916-921.
28. ŚLIWA-JÓŹWIK A., JÓŹWIK A., KOŁĄTAJ A., 2002 – Influence of exogenous glutathione (GSH), as stressfactor, on the activity of lysosome enzymes in some organs of mice. ***Archiv für Tierzucht*** 45, 307-314.
29. WITEK B., KOŁĄTAJ A., 1998 – The influence of selection on reaction to stress in mice V. The effect of starvation and glutathione injection on the activity of N-acetyl-beta-glucosaminidase and the level of glutathione in the liver. ***Journal of Animal Breeding and Genetics*** 115, 227-232.

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Aktywność układu lizosomowego we frakcjach komórkowych nerki myszy pod wpływem zredukowanego glutationu (GSH)

S t r e s z c z e n i e

Celem pracy było zbadanie, czy dootrzewnowe iniekcje zredukowanego glutationu (GSH) wpływają na aktywność enzymów lizosomowych w lizosomowej, mikrosomowej i cytozolowej frakcji komórkowej nerki myszy. Zwierzęta utrzymywano w warunkach standardowych z pełnym dostępem do paszy i wody. Myszy grupy doświadczalnej (n=10) otrzymały w ciągu 7 dni 14 dootrzewnowych iniekcji GSH, po 100 μg GSH/g masy ciała w 0,9% NaCl. Myszom grupy kontrolnej (n=10) podawano wyłącznie 0,9% NaCl. We frakcjach komórkowych nerki oznaczono aminopeptydazę alanylową, aminopeptydazę leucynową, katepsynę D i L, kwaśną fosfatazę i N-acetylo-β-D-glucosaminidazę. Najwyższą aktywność enzymów po iniekcjach GSH zaobserwowano we frakcji lizosomowej. Przypuszcza się, że wzrost aktywności badanych enzymów lizosomowych jest wynikiem adaptacyjnych zmian we frakcjach komórkowych, które wynikają z antyoksydacyjnych właściwości GSH.