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Effect of morphine on activity of five selected lysosomal enzymes in liver and kidneys of mice

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The experiment was carried out on 90 Swiss male mice divided into 9 groups (n=10). Over 4, 10 and 14 days mice of three control groups (I-III) were injected with 250 µl 0.9% NaCl solution daily, and those from six experimental groups (A-F) with 250 µl 0.9% NaCl solution containing 20 or 30 mg morphine hydrochloride per kg body weight. The injections were given intramuscularly once a day between 9:00-10:00 a.m. for 4, 10 and 14 days.
In the lysosomal fraction of the liver and kidney the activities of acid phosphatase, lysosomal esterase, β-glucuronidase, β-galactosidase, and β-N-acetyl-hexosaminidase were estimated. Morphine increased the activity of all examined enzymes except EL, which activity was statistically proven to decrease in liver and kidneys after 10 days morphine administration in both doses.

KEY WORDS: kidney / liver / lysosomal enzymes / mice

Since more than 200 years pharmacological action of morphine has been associated mostly with phenomenon of pain and analgesia [Savage 1999, Sternberg and Ridgway 2003]. It is assumed that morphine is the strongest painkilling agent which does not affect consciousness. Among other things morphine causes heart beat and breath acceleration, body temperature rise, blood pressure decrease as well as weakening of many functions of the immunological system [Watterson *et al*. 2004, Lötsch 2005, Sharp 2006].

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Since a long time pioneering studies have been associating morphine performances with stress reaction [Selye 1936]. The issue seemed to be particularly interesting due to specific role that lysosomes play in cells [Duran-Reyes *et al*. 1995, Cuervo and Dice 2000, Kołątaj *et al*. 2001, Bagshaw *et al*. 2005]. Their role, with reference to adaptive processes elicited by an action of opiates, particularly morphine, remains unexplained. Thus, the aim of this experiment was to identify an impact of two doses of morphine and time of injections on activity of the chosen lysosomal enzymes in liver and kidneys of mice.

Material and methods

The research has been conducted on 90 male Swiss mice at the age 8-9 weeks, with mean body weight of 23.0±1.3 g, randomly chosen from about a thousand of individuals kept on the mice farm at the Polish Academy of Science Institute of Genetics and Animal Breeding, Jastrzębiec. Animals were maintained under the standard farm conditions, in an air conditioned room with 50%-60% relative humidity, 21-22°C, 12-hour light cycle, and fed *ad libitum* the commercial granulated feed with standard 16% protein content, with free access to water. The diets were formulated by the Institute of Physiology and Animal Nutrition of the Polish Academy of Sciences, Jabłonna.

Mice were divided into 9 groups (n=10) – 6 experimental (A, B, C, D, E, F) and 3 control (I, II, III). Once a day, between 9:00 and 10:00 a.m., over 4, 10 and 14 consecutive days, mice from experimental groups were injected intramuscularly with morphine hydrochloride (Morphinum hydrochloricum, Polfa, Warsaw). Mice from groups A, C and E were given 20, and those from B, D and F – 30 mg morphine per kg body weight (BW) always in 250 µl of 0.9% NaCl per kg BW. Mice from control groups (I, II, III) were injected with 250 µl of 0.9% solution of NaCl in a dose of 250 µl/mouse over 4, 10 and 14 days.

Four hours after the last injection of morphine hydrochloride or 0.9% solution of NaCl mice were decapitated and fragments of liver and kidneys were sampled immediately to be homogenized according to Beaufay [1972] and then centrifuged [Janetzky K-26D] during 8 minutes at 600 g. The obtained supernatant was centrifuged once again during 20 minutes at 20 000 g [Janetzky K-26D] and sediment, were suspended in 0.1% Triton X100. In so obtained supernatants of the lysosome fraction of the liver and kidneys, the activities of acid phosphatase (AcP, EC, 3.1.3.2) and lysosomal esterase (EL, EC 3.1.1.2) were estimated according Barrett and Heath [1977], and of β-glucuronidase (β-GlcUr, EC 3.2.1.31), β-galactosidase (β-Gal, EC 3.2.1.23) and β-N-acetyl-hexosaminidase (Hex, EC 3.2.1.52) according to Barrett [1972]. In the lysosomal supernatans of liver and kidneys a total protein level was determined according to Kirschke and Wiederanders [1984]. Activities of enzymes were expressed in nmol/mg protein/hour.

The statistical verification of results was based on program [SAS/STAT [1999-2001, User's Guide, SAS Institute Inc., Cary, NC, USA] and Origin (version 5.0, Microcal

Software Inc. Northampton, USA). Substrates for enzyme and protein determination were purchased from Sigma (St. Louis, USA). Experiment was conducted following the agreement of Committee for Ethics in the Animal Experimentation at the Institute of Genetics and Animal Breeding, Jastrzębiec (18/99).

Results and discussion

Lysosomes are involved in the system of adaptive reaction of organism, and they are the subject of physiological examinations [Roberts and Deretic 2008, Witek 2000, Witek *et al*. 2005, 2006]. Lysosomal enzymes play a significant role in cell regulatory processes by maintaining in cell's interior specific, adequate for its needs homeostatic system, enabling an adaptation to changing environmental conditions [Kumański 2009, Liga *et al*. 2008].

Table 1. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the liver of mice injected over 4 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
AcP	10.56	2.57	11.10^{NS}	3.04	105	11.92^{NS}	2.90	113
EL	6.23	1.03	5.23^{NS}	1.85	84	5.05^{NS}	0.84	81
β-GlcUr	2.22	0.54	2.51^{NS}	0.72	113	2.87*	0.70	129
β-Gal	2.42	0.65	3.01*	0.69	124	3.25**	0.52	134
Hex	3.97	0.13	4.29^{NS}	1.59	108	5.29**	1.27	133

*, **Statistically different from control at P≤0.05, P≤0.01, respectively.
ns – not significant.

Table 2. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the kidney of mice injected over 4 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
AcP	9.55	1.41	9.44^{NS}	0.94	99	9.77^{NS}	1.44	102
EL	6.15	1.49	5.36^{NS}	0.44	87	5.10^{NS}	1.23	83
β-GlcUr	1.72	0.18	1.86^{NS}	0.15	108	2.08*	0.22	121
β-Gal	1.94	0.34	2.25^{NS}	0.26	116	2.30^{NS}	0.64	118
Hex	2.85	0.30	3.40^{NS}	0.35	119	3.27^{NS}	0.34	114

*Statistically different from control at P≤0.05.
ns – not significant.

Morphine when injected over 4 days in doses 20 and 30 mg/kg BW (group A and B) did not lead to any statistically confirmed changes in the liver and kidney acid phosphatase activity (Tab. 1 and 2). However, after 10 days of application (group C and D) its activity increased to 120% and 126% of the control in the liver and to 121% in the kidney after 30 mg injections (Tab. 3 and 4). The differences occurred not significant after injections lasting 14 days – group E and F (Tab. 5 and 6).

Biological role of AcP is closely related to defensive functions of the organism. As it is known, increased activity of AcP is observed in patients with prostatic, intestine and breast cancer, in primary neoplasm and metastases to bones, osteoporosis, liver affections, primary hyperfunction of parathyroid glands, coronary attack, thrombophlebitis and in patients with neoplastic lesions of reticuloendothelial system [Woźniak *et al*. 2002]. Increase in activity of acid phosphatase observed in liver after

Table 3. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the liver of mice injected over 10 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
AcP	11.25	3.67	13.50*	2.11	120	14.20*	1.84	126
EL	7.24	2.24	4.64**	0.69	64	3.78**	0.90	52
β-GlcUr	2.40	0.78	3.15**	0.43	131	4.21**	1.01	176
β-Gal	2.49	0.53	2.98^{NS}	0.60	119	3.08*	0.73	124
Hex	3.54	1.44	4.93**	1.33	139	5.53**	0.72	156

*, **Statistically different from control at P≤0.05 and P≤0.01, respectively.
ns – not significant.

Table 4. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the kidney of mice injected over 10 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
AcP	9.02	1.61	10.48^{NS}	1.83	116	10.91*	1.56	121
EL	6.85	0.84	4.58**	0.98	67	4.15**	0.34	61
β-GlcUr	1.80	0.26	2.23*	0.29	124	2.51**	0.73	139
β-Gal	2.04	0.27	2.55*	0.20	125	2.40^{NS}	0.67	117
Hex	3.11	0.81	3.42^{NS}	0.75	110	4.14**	0.65	133

*, **Statistically different from control at P≤0.05, P≤0.01, respectively.
ns – not significant.

Table 5. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the liver of mice injected over 14 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
AcP	12.1	1.91	14.25^{NS}	2.94	117	12.8^{NS}	1.84	106
EL	6.95	1.04	6.30^{NS}	2.31	91	5.82^{NS}	0.67	84
β-GlcUr	2.11	0.41	2.48^{NS}	0.40	118	2.41^{NS}	0.70	114
β-Gal	2.33	0.47	2.51^{NS}	0.44	108	2.40^{NS}	0.34	103
Hex	4.32	1.17	4.50^{NS}	0.49	104	4.44^{NS}	0.67	103

ns – not significant.

Table 6. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the kidney of mice injected over 14 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
AcP	9.58	1.68	10.91^{NS}	3.09	114	8.80^{NS}	1.23	92
EL	6.49	1.39	6.08^{NS}	0.43	94	5.32^{NS}	1.21	82
β-GlcUr	1.90	0.25	2.20^{NS}	0.40	116	2.15^{NS}	0.07	113
β-Gal	1.87	0.22	2.13^{NS}	0.24	114	1.97^{NS}	0.58	105
Hex	3.50	0.77	3.99^{NS}	0.41	114	3.16^{NS}	0.31	90

ns – not significant.

morphine administration, and proved statistically after 10 days of its performance, is probably a past-effect of accelerated rate of glycation metabolites phosphorylation and demand for providing the process with more phosphoric compounds. Augmented activity of AcP in kidneys may be related to increase in dephosphorylation of protein degradation products [Iwata *et al*. 1999].

Morphine administration in the doses of 20 and 30 mg/kg BW (group C and D) induced significant decrease in EL activity in relation to control (Tab. 3 and 4) in liver (to 64% and 52%) and kidneys (to 67% and 61%) only in the case of injections continued over 10 days. Main role of EL lies in participating in β-oxidation of long-chain fatty acids and degradation of fatty acids esters [Duee *et al*. 1985]. It is surmised that activity of EL may be related to formation of free radicals or decrease of antioxidant activity in oxidative stress [Li *et al*. 2007]. Advanced oxidation reactions evoke augmented lipid peroxidation, especially of membrane ones, what changes activity of phospholipase A2 and pace of arachidic acid pathway, and, as a consequence, leads to an increase in activity of cyclooxygenase (COX) and lipooxygenase. Bhat *et al*. [2004] noticed the morphine's

capability to induce oxidative stress, which, as we know, leads to destabilization of lysosomal membranes, what may result in the release of EL into a cytosol – observed after 10 days in form of significant decrease of EL activity in lysosomes.

The β-GlcUr activity increased statistically in liver and in kidneys after 10 days of morphine administration in doses of 20 and 30 mg/kg BW – group C and D (Tab. 3 and 4) as well as after 4 days of injections with doses of 30 mg/kg BW (Tab. 1 and 2). However, there was no change observed after 14 days. β-GlcUr is an exoglycosidase typical of mammalian tissues. High activity of β-GlcUr characterizes cells of endometrium, bowels, epididymes, liver, kidneys, salivary glands, placenta, spleen and leukocytes, while low activity is typical for brain. Alterations of β-GlcUr activity are observed in patients with hepatocirrhosis, pyelonephritis, diabetes, malignant tumours of stomach and central nervous system, leukaemia and anaemia [De Graaf *et al*. 2002]. Increased activity of β-GlcUr in liver and kidneys may suggest that the enzyme accelerated degradation of natural and synthetic β-D-glucuronides into free glucuronic acid [Kroemer and Klotz 1992]. Krumholz *et al*. [2001] demonstrated augmentation of the activity of β-GlcUr in leukocytes after administration of painkilling agents like ketamine, fentanyl and morphine.

β-Gal activity after injections of morphine in doses of 20 and 30 mg/kg BW (group A and B) for 4 days in the liver (Tab. 1) and in doses of 30 mg/kg BW (group D) for 10 days (Tab. 3) increased, but in kidneys β-Gal activity increased after 10 days only of 20 mg/kg BW dose. β-Gal is popularly branded as lactase commonly present in mammals' organs. The greatest activity of β-Gal has been demonstrated in lysosomal and cytosolic fraction of liver and lysosomal fraction of kidney [Hall *et al*. 1997]. Variation in β-Gal after morphine administration has already been observed in encephalon, unlike in liver and kidneys. Boundy *et al*. [1998] studied impact of chronic morphine and cocaine application on activity of TH gene promoter in mice and demonstrated that morphine practically doubles the activity of β-Gal in *nucleus coeruleus*. Increase of β-Gal activity in liver and kidneys after morphine application may be associated with acceleration of metabolism of glycosaminoglycans with participation of the alkaloid that happens in these glands.

The Hex activity after 4 days on a dose of 30 mg morphine per kg BW (group B) caused significant increase (to 133% of control) only in liver (Tab. 1), while injected for 10 days with doses of 20 and 30 mg/kg BW – group C and D (Tab. 3) – in liver (to 139% and 156%), and in kidneys in case of 30 mg/kg BW (group D) doses to 133% (Tab. 4). Hex may be used in diagnostics as a marker of some diseases, including malignant leukaemia, as well as in early detection of kidneys damage caused by toxic compounds performance [Izagirre *et al*. 2009]. The greatest activity of Hex can be observed in lysosomal fraction of liver, kidneys, spleen and leukocytes [Iwata *et al*. 1999]. Kharasch *et al*. [1997] revealed considerable augmentation of Hex activity in uriniferous tubules in rats, burdened with anaesthetics like sevoflurane and isoflurane. The glycosidase in question is a biomarker responsive for cells damages and necrosis [Price 1992]. Thus, under these conditions, activity of Hex should increase. Morphine

applied overly and for a longer time may indeed induce necrosis in a laden organism. Also Myśliwiec *et al*. [2006] regarded Hex as a marker responsive for necrosis-explained augmentation of activity of Hex by a development of diabetic retinopathy and nephropathy. In a diabetic nephropathy development process, there is an important role played by degradation of glycosaminoglycans, whose metabolites are discharged with urine. This is why considerable augmentation of Hex activity in renal tissue is highly possible. It can be suggested that increase of Hex activity in liver of the examined mice was, in all probability, related to augmenting after morphine administration need for Hex catalytic activity, indispensable for accelerated degradation of suitable glycosides accumulated, among other things, in autophagy vacuoles.

The results of the research presented here show that morphine administered daily to mice at doses of 20 and 30 mg/kg BW over 4, 10 and 14 days turned out to be a modificator of activity of almost all lysosomal hydrolases examined. It seems that augmentation of activity of lysosomal enzymes, excluding EL, may be related to an acceleration of rate of the degradation processes in liver and kidneys. If forthcoming studies will demonstrate that morphine beside analgesic properties can perform as a factor hastening the tempo of metabolic conversions, it may be used as one of the metabolic regulators.

It is interesting, that in the animals to which the morphine was administered over a long time (group E and F) there were not the significant differences. It may be assumed that numerous morphine injections gave rise to the resistance to this alkaloid in injected mice. It is a known phenomenon in human medicine.

REFERENCES

1. BAGSHAW R.D., MAHURAN D.J., CALLAHAN J.W., 2005 – Lysosomal membrane proteomics and biogenesis of lysosomes. ***Molecular Neurobiology*** 32, 27-42.
2. BARRETT A.J., 1972 – Lysosomal enzymes. In: Lysosomes, a Laboratory Handbook". Ed. J.T. Dingle, North-Holland Publishing Company, Amsterdam, New York, Oxford, 46-135.
3. BARRETT A.J., HEATH MF., 1977 – Lysosomal enzymes. In: Lysosomes. a Laboratory Handbook. Ed. J.T. Dingle, North-Holland Publishing Company, Amsterdam, New York, Oxford, 19-145.
4. BEAUFAY H., 1972 – Methods for isolation of lysosomes. In: Lysosomes, a Laboratory Handbook. Ed. J.T. Dingle, North-Holland Publishing Company Amsterdam 1972 1-45.
5. BHAT R.S., BHASKARAN M., MONGIA A., HITOSUGI N., SINGHAL P.C., 2004 – Morphine-induced macrophage apoptosis: oxidative stress and strategies for modulation. ***Journal of Leukocyte Biology*** 75, 1131-1138.
6. BOUNDY V.A., GOLD S.J., MESSER C.J., CHEN J., SON J.H., JOH T.H., NESTLER E.J., 1998 – Regulation of tyrosine hydroxylase promoter activity by chronic morphine in TH9.0-LacZ transgenic mice. ***The Journal of Neuroscience*** 18, 9989-9995.
7. CUERVO A.M., DICE J.F., 2000 – When lysosomes get old. ***Experimental Gerontology*** 35, 119-131.
8. DUEE P.H., PEGORIER J.P., MANOUBI L., HERBIN C., KOHL C., GIRARD J., 1985 – Hepatic triglyceride hydrolysis and development of ketogenesis in rabbits. ***American Journal of Physiology*** 249, E478-E484.
9. DURAN-REYES G., GONZALES-MACIAS G., HICKS I.J., 1995 – Lysosomal system in hormonal mechanisms. ***Ginecologia Obstetricia de Mexico*** 63, 68-73.

10. DE GRAAF M., BOVEN E., SCHEEREN H.W., HAISMA H.J., PINEDO H.M., 2002 – Beta-glucuronidase-mediated drug release. ***Current Pharmaceutical Development*** 8, 1391-1403.
11. HALL J.C., JACOBETZ D.R., LA MARCHE M.D., KOCHINS J., TUBBS C.E., 1997 – Purification, characterization and expression of rat epididymal β-D-galactosidase. ***Biochemistry and Molecular Biology International*** 42, 443-451.
12. IWATA T., UCHIDA S., HORI M., SAKAI K., TOWATARI T., KIDO H., 1999 – Human parathyroid hormone increases urinary excretion of lysosomal enzymes in rats. ***Life Science*** 65, 1725-1732.
13. IZAGIRRE U., ANGULO E., WADE S.C., GWYNN I., MARIQOMEZ I., 2009 – Beta-glucuronidase and hexosaminidase are marker enzymes for different compartments of the endo-lysosomal system in mussel digestive cells. ***Cell and Tissue Research*** 335, 441-454.
14. KHARASCH E.D., FRINK E.J., ZAGER R., BOWDLE T.A., ARTRU A., NOGAMI W.M., 1997 – Assessment of low-flow sevoflurane and isoflurane effects on renal function using sensitive markers of tubular toxicity. ***Anesthesiology*** 86, 1238-1253.
15. KIRSCHKE H., WIEDERANDERS B., 1984 – Methoden zur Aktivitätsbestimmung von Proteinasen. Martin-Luther-Universität Halle-Wittenberg Wissenschaftliche Beitrage Halle/Salle 11-17.
16. KOŁĄTAJ A., ŚLIWA-JÓŹWIK A., JÓŹWIK A., 2001 – The lysosomal cell complex as a stress response indicator. ***Animal Science Papers and Reports*** 3, 177-192.
17. KROEMER H.K., KLOTZ U., 1992 – Glucuronidation of drugs. A re-evaluation of the pharmacological significance of the conjugates and modulating factors. ***Clinical Pharmacokinetics*** 23, 292-310.
18. KRUMHOLZ W., WEIDENBUSCH H., MENGES T., KELLER G., HEMPELMANN G., 2001 – The influence of intravenous anesthetics on the activity of enzymes released from polymorphonuclear leucocytes in vitro. ***European Journal of Anesthesiology*** 18, 151-158.
19. KUMAŃSKI K., 2009 – Influence of vitamin A and restricted protein in food on lysosomal enzyme activity in the mouse liver and kidney. Edited and published by the Warsaw Agricultural University (Wydawnictwo Naukowe SGGW**),** Warszawa, 1-76.
20. LI Y., QIN Y., LI H., WU R., YAN C., DU H., 2007 – Lysosomal acid lipase over-expression disrupts lamellar body genesis and alveolar structure in the lung. ***International Journal of Experimental Pathology*** 88, 427-436.
21. LIGA A.B., UKHINA T.V., SHIMANOVSKI N.L., 2008 – Activity lysosomal enzymes in rat skin fibroblasts after treatment with progesterone and new gestagen ABMP. ***Bulletin of Experimental Biology and Medicine*** 145, 44-46.
22. LÖTSCH J., 2005 – Pharmacokinetic-pharmacodynamic modeling of opioids. ***Journal of Pain and Symptom Management*** 5, S90-S103.
23. MYŚLIWIEC M., ZORENA K., BALCERSKA A., MYŚLIWSKA J., LIPOWSKI P., RACZYNSKA K., 2006 – The activity of N-acetyl-beta-D-glucosaminidase and tumor necrosis factor-alpha at early stage of diabetic retinopathy development in type 1 diabetes mellitus children. ***Clinical Biochemistry*** 39, 851-856.
24. PRICE R.G., 1992 – Measurement of N-acetyl-beta-glucosaminidase and its isoenzymes in urine: methods and clinical applications. European Journal of Clinical Chemistry and Clinical Biochemistry: ***Journal of the Forum of European Clinical Chemistry Societies*** 30, 693-705.
25. ROBERTS E.A., DERETIC V., 2008 – Autophagic proteolysis of long-lived proteins in nonliver cells. ***Methods in Molecular Biology*** 445, 111-117.
26. SAVAGE S.R., 1999 – Opioid use in the management of chronic pain. ***Medical Clinics North America*** 83, 761-786.
27. SELYE H., 1936 – A Syndrome Produced by Diverse Noxious Agents. ***Nature*** 138, 32-34.
28. SHARP B.M., 2006 – Multiple opioid receptors on immune cells modulate intracellular signaling. ***Brain Behavior and Immunity*** 20, 9-14.

29. STERNBERG W.F., RIDGWAY C.G., 2003 – Effect of gestational stress and neonatal handling on pain, analgesia, and stress behavior of adult mice. ***Physiology and Behavior*** 78, 375-383.
30. WATTERSON G., HOWARD R., GOLDMAN A., 2004 – Peripheral opioids in inflammatory pain. ***Archives of Disease in Childhood*** 89, 679-681.
31. WITEK B., 2000 – Fizjologia reakcji stresowej i model lizosomalnego układu degradacyjnego (Physiology of stress reaction and model of lysosomal degradation system) In Polish. Edited and published by Warsaw Agricultural University (Wydawnictwo Naukowe SGGW), 1-60.
32. WITEK B., OCHWANOWSKA E., RAFAY J., KOŁĄTAJ A., CHRENEK P., SUVEGOVA K., JURCIK R., SIROTKIN A., DARLAK K., 2005 – Effect of ghrelin on activities of some lysosomal hydrolases in rabbits. ***Neuroendocrinology Letters*** 26, 397-400.
33. WITEK B., GRANICZKA A., OCHWANOWSKA E., KOŁĄTAJ A., 2006 – Aktywność enzymów lizosomowych w wątrobie i nerkach myszy po iniekcjach glukagonu. ***Medycyna Weterynaryjna*** 62, 717-719.
34. WOŹNIAK A., DREWA T., ROZWODOWSKA M.. DREWA G., LAMBRECHT W., WIŚNIEWSKA I., 2002 – Activity of some lysosomal enzymes in serum and in tumors of patients with squamous cell lung carcinoma. ***Neoplasma*** 49, 10-15.