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CHANGES IN ACTIVITY OF ACID HYDROLASES IN TISSUES OF WILD PHEASANTS INDUCED BY HEAVY METALS

ZMIANY AKTYWNOŚCI KWAŚNYCH HYDROLAZ W TKANKACH DZIKICH BAŻANTÓW WYWOŁANE PRZEZ METALE CIĘŻKIE

Abstract: The aim of the study was to determinate the activity of chosen acid hydrolases in tissues of pheasants (*Phasianus colchicus* L.) and first attempts to use these enzymes as biomarkers of the effect of birds exposure to heavy metals in the environment. The samples of liver, kidneys and testes were collected from wild pheasants shot in the Podkarapacie region in contaminated (n = 5) and clear (n = 5) areas. Tissues were examined for the cadmium and lead concentrations by AAS method, as well as the activity of five hydrolases.

The major site of cadmium accumulation were kidneys, the levels of Cd found in liver and testes were by 5-fold and 30-fold lower ($P < 0.05$), respectively. Similarly, Pb concentrations observed in the liver and testes were lower than in kidney, by 1.5-fold and 6-fold, respectively. The presence of four glycosidases and arylsulphatase in all examined tissues was demonstrated and N-acetyl-β-D-glucosaminiadase (NAG) was identified as the most active enzyme in all studied tissues. Significant differences ($P < 0.05$) were observed only in the level of NAG activity in liver of pheasants inhabiting the contaminated area as compared to the birds from the clear region (control group). We conclude that the determination of NAG in birds organs can be used as biomarker of environmental exposure to cadmium.

Keywords: pheasant, heavy metals, biomarker, acid hydrolases

Introduction

Increasing pollution and environmental degradation are difficult to avoid because of the intense, constant development of many industries and the energy sector [1]. The presence of heavy metals cannot be found in the heavily industrialized regions only, but

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also in natural and agricultural ecosystems. Among the pollutants prominent place is occupied by heavy metals such as lead, cadmium and mercury.

Determination of environmental metals does not reflect the level of their impact on health and biochemical changes in the body of animals or people (the effects of exposure) [2]. Therefore, in recent years there has been a growing interest in using bioindicators for monitoring environmental pollution with heavy and toxic metals and their toxic effect for living organism [3, 4]. In this respect, pheasant play a valuable role in environmental monitoring of their vulnerability to human exploitation, sensitivity to habitat degradation, and central position in the food web [5].

The kidneys and liver are the organs affected by accumulation of toxic heavy metals in the body of animals and humans. Heavy metals originating from the air, food or water, can change the distribution of lysosomal enzymes in an intra-and extracellular fluids [4, 6–8]. Simultaneous administration of cadmium and lead caused enhancement of degenerative changes in proximal tubule cells [6, 9]. These observations suggest that the combined administration of metals causes renal damage that appears to be additive [9]. Therefore, an important issue is the search for enzymatic markers of early warning against the effects of exposure to the toxic heavy metals.

N-acetyl-β-D-glucosaminidase (NAG), one of the lysosomal enzyme, is also present in small amounts in the microsomal fraction of kidney tubule cells which activity is increased by cadmium intoxication [4, 6, 10]. According to the dose of cadmium, the number and size of lysosomes are increased, indicating a subtile cell damage. Therefore, NAG is a recognized marker of the cytotoxic effects of heavy metal compounds [11–13]. The increased levels of urinary N-acetyl-β-D-glucosaminidase observed in cadmium-exposed workers could be used as biomarkers for suggesting preventive measure [14, 15].

Besides NAG, other lysosomal enzymes such as: β-galactosidase, α- and β-mannosidase, arylsulphatase were also used for the diagnosis of multiple tissues damage [12, 16–18]. Since then, the presence of glycosidases and arylsulphatase has been demonstrated only in reproductive tissues and semen of pheasant [19].

In this work the suitability of using chosen acid hydrolases from pheasants tissues as biomarkers of environmental exposure of birds to heavy metals pollution was studied.

Materials and methods

1-year old male pheasants (n = 10) were shot by hunters using lead shots in February (the mean body weight 1.20 ± 0.26 kg). Birds originated from the urban area of Rzeszow (group A, n = 5), and from clear region (group B, n = 5) both localized in South-Eastern Poland (Podkarpacie region). Tissue samples were collected immediately and transported on ice to the laboratory. They were stored at 20 ^{o}C until analyzed. Heavy metals and enzyme assays were performed on livers, kidneys and testes of birds.

Cadmium (Cd) and lead (Pb) concentrations were assayed by AAS method using Hitachi Z-2000 (Japan) spectrophotometer equipped with a graphite furnace after prior microwave mineralization in HNO_3 (Speedwave Four, Berghof, German). The metal concentrations were expressed on a wet weight basis (mg/kg).

The activity of five lysosomal enzymes: N-acetyl-β-D-glucosaminidase (NAG; EC 3.2.1.30), α- and β-D-mannosiadase (α-MAN; EC 3.2.1.24, β-MAN; EC 3.2.1.25), β-galactosidase (β-GAL; EC 3.2.1.23) and arylsulphatase (ARYL; EC 3.1.6.1) was determined. Enzymatic activities were tested in the supernatant of tissues homogenates (10% w/v in a saline with 0.1% Triton additive), according to the method of Barrett and Heath [20]. Arylsulphatase activity assay was performed according to the Baum method [21]. The synthetic substrates (Sigma, USA) in optimum pH were used and the absorbance of released 4-nitrophenol was measured at 400 nm (for ARYL 4-nitrocatechol at 515 nm). The enzymatic activity was expressed in units (U) [mmol $\cdot$ cm^{-3} $\cdot$ min^{-1}]. Determination of the protein content of the tissues was performed by the Lowry method [22] using bovine serum albumin as a standard.

Differences between tissues were assessed using Kruskal-Wallis and Mann-Whitney U-tests ($P < 0.05$). Coefficients of correlation were calculated using Spearman's correlation analysis.

Results and discussion

Enhanced levels of both heavy metals were observed mainly in kidneys, following by liver and testes (Table 1). The levels of Cd found in liver and gonads were 5-fold and 30-fold lower ($P < 0.05$) than observed in kidneys, respectively. Similarly, Pb concentrations observed in the liver and testes were respectively 1.5-fold and 6-fold lower than in kidneys ($P > 0.05$). There was no synergistic relationship between lead and cadmium tissue concentration ($P > 0.05$).

Table 1

Concentrations of cadmium and lead (means ±SD; mg/kg wet weight) in tissues of pheasants derived from urbanized area (Group A) and clear region (Group B)

Heavy metal	Tissues		
	Liver	Kidneys	Testes
		Cadmium	
Total	0.67 ± 0.84^{a}	$3.33 \pm 2{,}76^{b}$	0.07 ± 0.16^{c}
Group A	1.08 ± 0.80*	5.73 ± 0.69*	0.11 ± 0.13
Group B	0.25 ± 0.08	0.92 ± 1.25	0.03 ± 0.18
		Lead	
Total	0.17 ± 0.03	0.25 ± 0.04	0.04 ± 0.06
Group A	0.16 ± 0.13	0.24 ± 0.04	0.06 ± 0.08
Group B	0.18 ± 0.04	0.17 ± 0.04	0.02 ± 0.03

* – significant differences between A and B groups ($P < 0.05$); $^{a, b, c}$ – significant differences between concentrations of heavy metals in tissues ($P < 0.05$).

The cadmium concentration in liver and kidneys of pheasants coming from urbanized area (group A) was significantly higher ($P < 0.05$), but great individual variability in Cd level was observed (Table 1). This tendency was not occurred for lead content in all tissues studied.

These results are in agreement with other authors finding that birds from urban areas have higher tissues concentrations of both lead and cadmium [23, 24]. Moreover, the elevated levels of cadmium damage mainly the kidneys of birds and mammals, although the male reproductive system was also affected. Probably this metal is co-responsible for a decrease in the number of population of Mallard observed in wetlands in North-Western Poland [25].

Table 2

The specific activity of hydrolytic enzymes [mU/mg protein] in tissues of shot pheasants (*Phasianus colchicus* L.) (n = 10)

Enzyme	Activity of hydrolytic enzymes [mU/mg protein]		
	Liver	Kidneys	Testes
		NAG	
mean ±SD	348.54 ± 70.42[a]	744.21 ± 115.18[b]	1104.04 ± 203.12[c]
min.	265.62	530.72	907.28
max.	435	861.92	1413.64
variability [%]	20.2	15.5	18.4
		β-GAL	
mean ±SD	36.35 ± 8.44[a]	43.32 ± 6.37[a]	233.91 ± 56[b]
min.	21.60	35	161.8
max.	47.80	50.87	295.86
variability [%]	23.2	14.7	23.9
		α-MAN	
mean ±SD	44.71 ± 8.04[a]	52.17 ± 7.08[a]	34.87 ± 7.87[a]
min.	34.14	41.25	25.87
max.	54.09	62.52	47.1
variability [%]	18	13.6	22.6
		β-MAN	
mean ±SD	18.55 ± 3.98[a]	18.3 ± 2.82[a]	27.35 ± 3.82[a]
min.	14.21	14.89	23.97
max.	24.66	23.04	34.5
variability [%]	21.5	15.4	14
		ARYL	
mean ±SD	24.26 ± 6.42[a]	22.4 ± 4.43[a]	65.63 ± 16.38[b]
min.	16.58	17.28	41.33
max.	31.84	29.26	84.55
variability [%]	26.5	19.8	25

[a, b, c] – significant differences between activity of enzymes in tissues ($P < 0.05$).

Research carried out by Toman et al [5] showed that the cadmium concentrations in kidneys and liver increased significantly in adult pheasants. The metal was accumulated especially in kidneys of the adult pheasants and reached levels up to 9.64 mg/kg wet weight after the 3 months daily cadmium intake in drinking water. Similarly, the highest cadmium concentrations were also found in the kidneys (up to 117.6 mg/kg dry weight) and liver (37.1 mg/kg dry weight) of voles fed with cadmium [2]. Moreover, histological examination of the tissues revealed some pathological changes in the structure of kidneys, liver and testes of voles after chronic exposure.

Among the analyzed enzymes NAG showed the highest activity in all examined organs of pheasants while the lowest value was marked for β-MAN (Table 2). The activity of enzymes in examined tissues of birds declined in the following order:

liver: NAG > α-MAN > β-GAL > ARYL > β-MAN,
kidneys: NAG > α-MAN > β-GAL > ARYL > β-MAN,
testes: NAG > β-GAL > ARYL > α-MAN > β-MAN.

The highest activity of enzymes was noted in the testes of birds, excluding α-MAN. Significant differences in the activity of hydrolytic enzymes ($P < 0.05$) were determined for NAG in the all studied organs of birds and for ARYL and β-GAL in the testes (Table 2). The ratio between NAG and other studied enzymes activity was much higher in liver and kidneys tissues. These results are similar to earlier research [19, 26]. In all studied organs, the lowest activity was found for β-MAN and was lower about 59% in liver, 65 % in kidneys, 22% in testes than the activity of a-MAN. A similar relationship was observed for the activity of these enzymes in testes of duck during breeding season [26].

Significant decrease ($P < 0.05$) in the level of NAG activity was observed in the liver of pheasants inhabiting the contaminated area (group A) as compared to the clear region (Fig. 1a). An adverse effect for ARYL in testes was demonstrated ($P < 0.05$) (Fig. 1b).

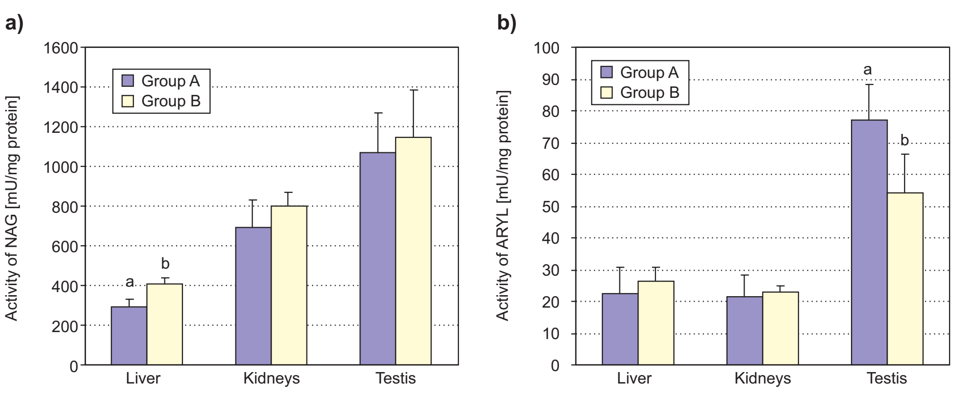

Fig. 1. The effect of environmental pollution on the activity of NAG (a) and ARYL (b) (mU/mg protein) in tissues of pheasants derived from urbanized area (Group A) and clear region (Group B); [a, b] – significant differences ($P < 0.05$)

Other studied enzymes, such as: β-GAL, α-MAN, β-MAN and ARYL were characterized by lower activity (Table 2). Their level in the liver and kidneys was

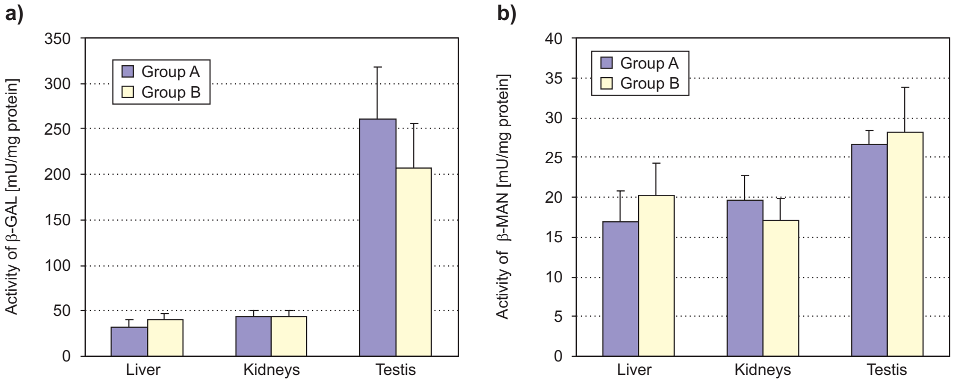

Fig. 2. The effect of environmental pollution on the activity of β-GAL (a) and β-MAN (b) (mU/mg protein) in tissues of pheasants derived from urbanized area (Group B) and clear region (Group A)

comparable and independent on the origin of birds (Fig. 1, 2a and 2b). The level of NAG activity was inversely correlated with the contents of cadmium and lead only in the liver (Table 3).

Table 3

Spearman correlation analysis of enzyme – metal interactions in pheasants tissues

Tissue/Metal	Enzyme				
	NAG	β-GAL	α-MAN	β-MAN	ARYL
Liver					
Cd	–0.71	–0.37	–0.43	–0.20	0.09
Pb	–0.77	–0.89	–0.31	–0.71	–0.54
Kidneys					
Cd	0.43	–0.26	–0.20	0.26	–0.37
Pb	0.12	0.23	0.38	0.46	0.38
Testes					
Cd	0.43	0.66	0.60	0.37	0.77
Pb	–0.31	0.37	0.94	0.14	0.54

Our findings are in agreement with study carried out by Bairati et al [11], who observed a significant decrease in activity of NAG and activity of glucuronidase with the maximum concentration of lead and manganese *in vivo* in blood plasma and *in vitro* in cultures of mitogen-activated lymphocytes.

In the opposite, Dzugan et al [12] showed an increase in the activity of NAG, β-MAN and ARYL in plasma of day-old chicks which indicated to damage kidneys and liver caused by toxicity of cadmium. Moreover, multiple researchers reported a positive correlation between excretion of cadmium ions and urinary NAG activities in humans environmentally exposed to Cd as well as in Cd-workers [10, 14, 15].

Many studies have confirmed the usefulness of NAG activity in the assessment of renal damage by impact of heavy metals such as cadmium and lead [8, 15, 27]. Brzoska et al [6] have used an experimental model of rats chronically exposed to cadmium. They observed, the increased urinary activities of NAG and its specific form (NAG-B), which were well correlated with early lesions in the main renal tubules. The enhanced excretion of enzyme due to Cd influence resulted from their leakage into cytoplasm via damage of lysosomal cellular membranes. The damage of the lysosomes and other cellular organelles, was demonstrated using well known biochemical markers of kidney status such as: low molecular weight serum protein in urine (α_1-microglobulin, β_1-microglobulin, retinol binding protein) and enzymes from the renal cortex (N-acetyl-β-D-glucosaiminidase) [28]. Therefore, the observed decrease in NAG activity in liver may be connected with excretion of lysosomal enzymes to blood.

Conclusions

It was firstly shown that cadmium and lead accumulation in tissues of pheasant affected the activity of the most active acid hydrolases NAG and this effect was significant for liver. The determination of NAG activity in pheasants liver can be suitable for monitoring of heavy metals in the environment. Due to a small birds population used in experiment it requires a further study.

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ZMIANY AKTYWNOŚCI KWAŚNYCH HYDROLAZ W TKANKACH DZIKICH BAŻANTÓW WYWOŁANE PRZEZ METALE CIĘŻKIE

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Abstrakt: Celem badań było oznaczenie aktywności wybranych kwaśnych hydrolaz w tkankach bażantów (*Phasianus colchicus* L.) oraz zastosowanie po raz pierwszy tych enzymów jako biomarkerów narażenia środowiskowego ptaków na metale ciężkie. Próbki wątroby, nerek oraz jąder zostały pobrane z bażantów odstrzelonych na Podkarpaciu w rejonie zurbanizowanym (n = 5) i ekologicznym (n = 5). Analizy tkanek obejmowały oznaczenie zawartości metali ciężkich (Cd, Pb) metodą AAS oraz aktywności kwaśnych hydrolaz.

Głównym narządem docelowym akumulacji metali ciężkich były nerki, zawartość kadmu w wątrobie oraz jądrach była niższa odpowiednio 5- i 30-krotnie. Podobną zależność zaobserwowano dla ołowiu, koncentracja tego metalu w jądrach i nerkach była niższa odpowiednio 1.5- i 6-krotnie. We wszystkich badanych tkankach stwierdzono obecność czterech glikozydaz i arylosulfatazy, przy czym najobfitszym źródłem enzymów były jądra, a najbardziej aktywnym enzymem we wszystkich tkankach była N-acetylo-β-D-glukozaminidaza (NAG). Wykazano istotne różnice ($P < 0.05$) w poziomie aktywności NAG w wątrobie bażantów bytujących na terenie zurbanizowanym w porównaniu do regionu ekologicznego. Aktywność NAG w tkankach ptaków może być przydatnym biomarkerem środowiskowego narażenia na kadm.

Słowa kluczowe: bażant, metale ciężkie, biomarker, kwaśne hydrolazy