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Variations of Glycolytic Kinases and Pentose-Phosphate Pathway Dehydrogenases in Response to Lead Accumulation in Hemopoietic Cells of Rock Doves (*Columba livia*)

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The glycolytic enzymes, hexokinase (HK, E.C. 2.7.1.1), phosphofructokinase (PFK, E.C. 2.7.1.11) and pyruvatekinase (PK, E.C. 2.7.1.40) regulate glycolysis in mammalian red blood cells. Moreover, 3-phosphoglyceratekinase (PGK, E.C. 2.7.2.3) is the catalyst for the first step of ATP recuperation in red blood cell glycolysis. Columba livia erythrocytes seem to have the same glycolytic regulatory and flux controlling enzymes (HK, PFK and PK), as do human and amphibian erythrocytes (Calopenopoulou et al., 1989). Glucose 6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, E.C. 1.1.1.44) are essential to the cytoplasmic production of NADPH. This reduced nucleotide is indispensable in the maintenance of the level of antioxidant glutathione (GSH) needed for both erythrocyte viability (Delgado et al. 1991) and the function of many enzymes whose activities increase during cell proliferation (Rao et al. 1984).

Lead causes serious damage in bone marrow and peripheral blood cells, interfering with and inhibiting cell proliferation in the bone marrow (Kowolenko et al. 1991). Lead inhibits δ-aminolevulinic acid dehydratase (δ-ALAD) in erythrocytes and bone marrow cells (González and Tejedor 1992), and produces dose-dependent decreases in GSH and GPDH (Sierra et al. 1989). The effects of lead on G6PDH activity differ in function of the tissues analyzed: Hacker et al. (1990) found a notable increase in pentose-phosphate pathway in liver while Cocco et al. (1991) reported a decrease.

The aims of the present study were to evaluate the activity levels of glycolytic kinases and dehydrogenases in the pentose-phosphate pathway in erythrocytes and bone marrow cells of control rock doves and to test whether lead accumulation would induce metabolic changes in the carbohydrate metabolism by affecting these regulatory enzymes. These enzymes were also studied in erythrocytes from male and female rock doves to study the sex incidence of the effect of lead.

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MATERIALS AND METHODS

Two groups of twenty adult doves (male and female) were kept in our laboratory located on the outskirts of Alcalá de Henares city (Spain). They were fed water and grain "ad libitum" which were free of lead contamination. Doves were weighed and a suitable dose of lead acetate (5 mg/Kg equivalent to 2.77 ppm of Pb in total body weight) was administered orally once a week, for one, two, three or four weeks. One week after the last dose, blood samples were extracted from the braquial vein, the doves were killed and the femur and tibia removed. Pools of bone marrow cells were isolated from the long bones as described by Tejedor et al. (1984), and both types of samples were hemolysed as described previously (Pinilla et al. 1987) and kept at 4°C.

Enzymatic activities (HK, PFK, PGK, PK, G6PDH and 6PGDH) were measured by the appearance or disappearance of NADH or NADPH (increase or decrease of absorbance at 340 nm) in a Simazu spectrophotometer at 30ºC. In each case, 25-50 uL of biological sample were mixed to a final volume of 3 mL with the following components, depending on the enzyme to be tested.

HK test mixture contained 100 mM Tris-Cl, pH 7.1, 0.33 mM EDTA, 6.7 mM $MgCl_2$, 20mM glucose, 2.7 mM ATP, 1 mM NADP+ and 0.5 U/mL glucose 6-phosphate dehydrogenase.

PFK test mixture contained 100 mM Tris-HCl pH 7.1, 0.3 mM EDTA, 3 mM MgCl2, 90 mM KCl, 0.15 mM NADH, 1.5 mM dithiothreitol, 1 mM phosphate, 3 mM F6P, 9 mM G6P, 1.5 mM ATP, 0.1 U/mL fructose diphosphate aldolase, 6 U/mL triose phosphate isomerase and 0.5 U/mL glycerol 3-phosphate dehydrogenase.

PGK test mixture contained 100 mM Tris-Cl buffer, pH 7.5, 0.5 mM EDTA, 6.2 mM $MgCl_2$, 5 mM ATP, 0.2 mM NADH, 4 U/mL of glyceraldehyde 3-phosphate dehydrogenase and 100 mM 3-phosphoglycerate.

PK was measured by a coupled NADH-LDH method. The assay mixture contained 50 mM Tris-HCl buffer, pH 7.5, 0.6 mM EDTA, 75mM KCl, 8 mM $MgCl_2$, 0.4 mM ADP, 0.2 mM NADH, 3 U/mL LDH and 1.5 mM PEP.

Dehydrogenases were determined in a double-step assay. The first cuvette contained 50 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.5 mM NADP, 0.6 mM G6P and 0.6 mM 6PG. The appearance of NADPH (an increase in absorbance at 340 nm) indicates the sum of activities of both G6PDH and 6PGDH. In the second step, G6P is omitted and the activity corresponding to 6PGDH is obtained. G6PDH activity is then calculated by subtracting 6PGDH activity from the one corresponding to the sum of both activities.

Protein was estimated according to Lowry et al. (1951), using bovine serum albumin as standard. Student's t test was applied to determine the statistical significance.

RESULTS AND DISCUSSION

The levels of glycolytic enzyme activities in rock dove cells, obtained from untreated control animals, are summarized in Table 1. Activities of all the enzymes were several folds higher in the bone marrow cells as compared to the erythrocytes. The ratio between activity levels in bone marrow cells and erythrocytes is 50 for HK, and ranges from 25 to 10 for PFK, PGK, PK and 6PGDH, and 4 for G6PDH. These ratios demonstrate the higher intensity of the metabolic activities in precursor cells in differentiation and proliferation processes as compared to mature cells. A similar relation has been reported in mammals (Nijhof et al. 1984; Shinohara et al. 1985).

Sex differences appear in these enzymes in erythrocytes (Table 1). Enzymatic activities are slightly higher in female than in male doves and the ratios for female to male activity are 1.4 for PK and PGK, and 1.05 for G6PDH.

When the kinase activities are compared among themselves in the three cell groups (Table 1) the high levels of PGK and PK, the kinases that control the glycolytic steps for ATP recuperation stand out. This result is in agreement with previous findings in mammalian erythroyd cells (Shinohara et al. 1985; Jansen et al 1985 and Jimeno 1988). In both erythrocytes and bone marrow cells, HK had the lowest activity of all the enzymes. It limits the glycolysis rate in human erythrocytes (Shinohara et al. 1985; Jansen et al. 1985; Forniani et al. 1986), and reticulocytes (Jansen et al., 1985) as well as in other tissues in other species, such as muscle and heart in hens and pigeons (Bloomstrad et al. 1983).

Table 1. Activity levels of glycolytic kinases and pentose phosphate pathway dehydrogenases in erythrocytes and bone marrow cells of control rock doves.

	Erythrocytes		Bone marrow cells
	Female doves	Male doves	Male doves
	(U/g Protein)	(U/g Protein)	(U/g Protein)
Kinases			
HK	0.38 ± 0.18	0.32 ± 0.01	15.7 ± 4.0 ***
PFK	2.74 ± 0.17	2.20 ± 0.54	29.5 ± 0.3 ***
PGK	55.00 ± 8.70	39.00 ± 2.10	721.0 ± 8.0 ***
PK	29.20 ± 4.70	20.05 ± 2.25	277.0 ±47.0 ***
Dehydrogenases			
G6PDH	6.6 ± 0.2	6.3 ± 1.8	26.5 ± 1.0 ***
6PGDH	1.3 ± 0.4	1.1 ± 0.02	27.0 ± 3.0 ***

N=5, values are expresed X±SE. Superscripts within columns denote statistical differences in enzymatic activity between erythrocytes and bone marrow cells:*** $p<0.001$, ** $p<0.01$.

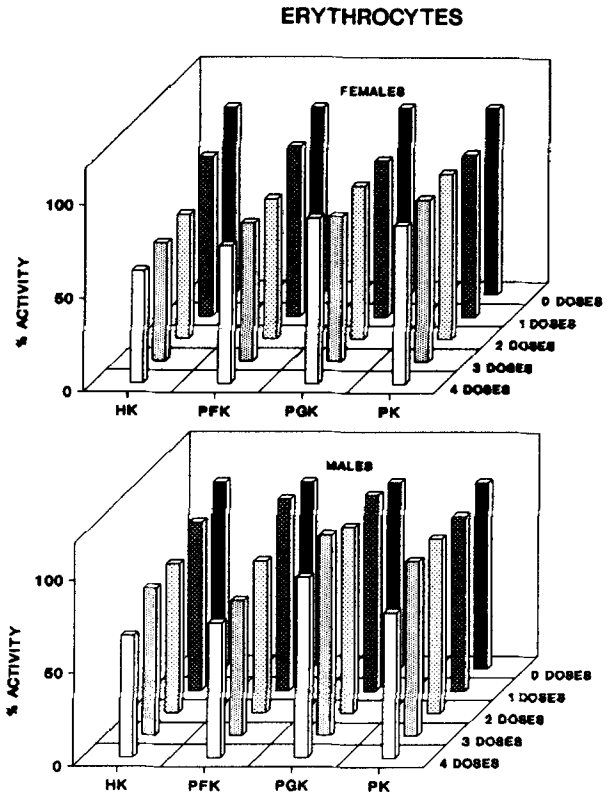

Figure 1. Variations of kinase activities in rock dove erythrocytes during lead treatment.

The activity of G6PDH and 6PGDH in rock dove erythrocytes (Table 1), is similar to data reported in ducks and chickens (Narasimhan and Baurm 1973). These authors have attributed the presence of high dehydrogenase activities in avian erythrocytes to the nucleated nature of these cells and the high blood glucose level in birds. Since 6PGDH has the lowest activity of the two dehydrogenases, in both male and female rock doves, this enzyme must be the one to limit the pentose-phosphate pathway rate in erythrocytes. Therefore the effect of the xenobiotic on these enzymes and particularly on 6PGDH could represent a serious damage on the essential NADPH production carried out in these cells.

The ratio of G6PDH/6PGDH activities denotes the rate of the pentose phosphate pathway and its variation indicates the influence of this pathway in different cells or tissues (Farnararo et al. 1980). The ratio is high in erythrocytes, 5.7 in males and 5.1 in females, but in bone marrow cells it is about 1, which means that 6PGDH activity shows the highest variability between these tissues and therefore this enzyme would have the key role in cotrolling NADPH requirement in both types of cells.

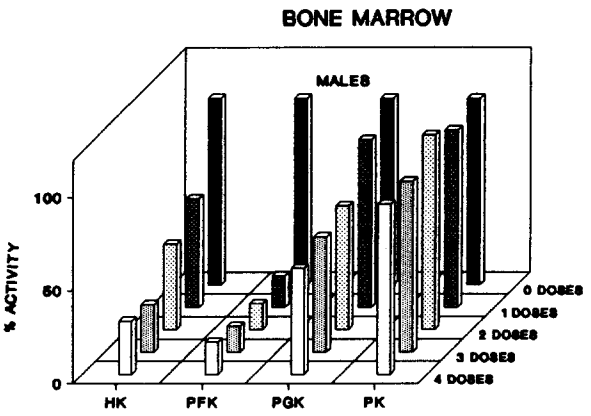

Figure 2. Variations of kinase activity values in bone marrow cells of male rock doves during lead treatment.

Generally speaking, lead inhibited each of the kinases to a different degree. The most affected kinase in the erythrocytes was HK (Fig. 1). Its levels decreased as low as 60% of the control values in females and 65% of the control values in males, after the fourth lead dose. The effect was similar in the activities of PFK and PK, both of which decreased more in erythrocytes from females than those from males, after the fourth lead dose, their values were about 80-75% of control activity. Activity of PGK was the least affected by lead accumulation and only a slight decrease was found in females. The greater susceptibility in female doves could be explained by the higher blood lead level in female as compared to male rock doves (Tejedor and González 1992). Since HK activity suffers the highest inhibitory effect from lead treatment and it is the key to the glycolytic rate in erythrocytes, its decrease would imply serious damage to the metabolic energy source in these cells.

Bone marrow cell kinase levels also decrease during lead treatment (Fig. 2), HK and PFK being the most markedly decreased activities. After the fourth lead dose, they only show 30% and 20%, respectively, of their control values (Fig. 2), with a gradual decrease for HK and a sharp decrease for PFK as soon as the first dose.

The PGK activity decreases gradually to 60% of control levels. PK is the least affected enzyme and its activity decreases only slightly (90% of controls) at the end of the lead treatment. Consequently, lead administration markedly reduces the bone marrow cell glycolytic rate by strongly decreasing the first two regulatory kinases involved in glycolysis. In addition, the marked decrease of PFK activity after the first lead dose (Fig. 2) signifies a severe reduction of glycolytic pathway activity from the level at the beginning of the lead treatment. This would lead to serious damage to the metabolic energy source in bone marrow

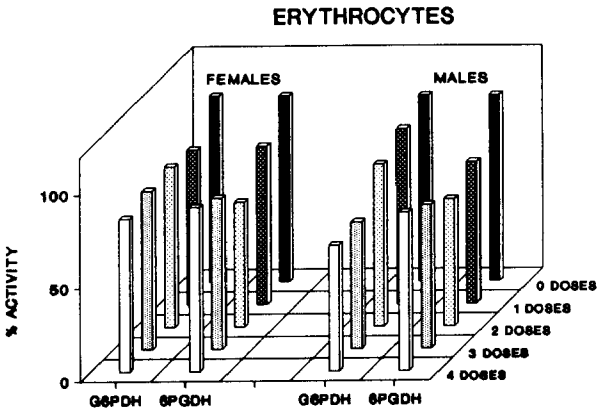

Figure 3. Variations of pentose phosphate pathway dehydrogenases in rock dove erythrocytes during lead treatment.

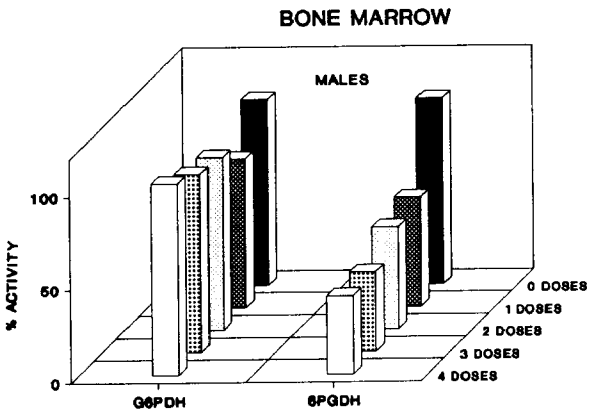

Figure 4. Variations of pentose phosphate pathway dehydrogenases in bone marrow cells of male rock doves during lead treatment.

cells, since these cells are in continuous differentiation and proliferation processes.

Rock dove erythrocyte 6PGDH activity decreases with the lead treatment (Fig. 3). This activity decreases during the two first doses in erythrocytes from both male and female doves down to 67% of control values (Fig. 3), but in the following doses, the activities increased, reaching 85% - 88%, respectively of control values.

The decrease and increase produced in erythrocyte 6PGDH activity during the lead treatment (Fig. 3) is similar to that found in bone marrow cell δ-ALAD activity (González and Tejedor 1992), and therefore an inductive response in the 6PGDH synthesis as a response to its inhibiton by lead could also be deduced here. Since this enzyme is the key to the pentose-phosphate pathway rate, its activity mantains the reducing power in these cells.

In male rock dove bone marrow cells, the G6PDH activity decreases to 80% of control value after the first lead dose (Fig. 4) and returns to control values with the last dose. On the contrary, the 6PGDH activity decreases after the first lead dose to 59% of the control values and remains depressed at the end of the treatment. These results differ from other data that show an increase in liver G6PDH and 6PGDH activities after lead treatment (Hacker et al. 1990) associated with lead biotransformation as well as liver cell proliferation. However, since the lead transformation capacity in bone marrow is lower than in liver and the cellular proliferation in bone marrow cells should be lowered by lead (Kowolenko et al. 1991), is seems logical that the G6PDH and 6PGDH activities would be decreased in bone marrow cells. The xenobiotic inhibits the 6PGDH enzyme and consequently decreases the reducing power of these cells, thus affecting cellular proliferation.

In summary, it is obvious that the lead influence on kinases in bone marrow cells is stronger than that found in the erythrocytes and results in a greater decrease of their capacity to obtain energy from ATP. Moreover, the inhibition of 6PGDH activity in bone marrow cells implies a serious loss of production of reducing power. The combination of these two lead effects will negatively affect the bone marrow cell biosynthesis and proliferation.

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