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In vitro Inhibition of Digestive Enzymes by Heavy Metals and Their Reversal by Chelating Agent: Part II. Lead Nitrate Intoxication

K. V. Sastry and P. K. Gupta
Department of Zoology, D.A.V. (P.G.) College, Muzaffarnagar, India

Introduction

Trace elements present in the body may be divided into essential and non-essential metals. The levels of essential metals are maintained by some biological processes where as those of non-essential metals is through external sources. Non-essential metals like lead, cadmium and mercury produce cumulative toxic effects if taken in small doses, and acute toxicity in higher doses (HARRISON et al. 1971). Toxic effects of non-essential metals and chemicals may result from their binding with biologically active constituents of the body such as lipid, amino acids, enzymes and proteins (PASSOW et al. 1961). Though the exact mode by which lead interferes with the physiological functions of brain, liver, kidney (BLACKWOOD et al. 1961) and digestive system (SASTRY & GUPTA, 1977a,b) is not known, alteration in the activities of certain enzymes has been observed in experimental lead poisoning (SASTRY & GUPTA, 1977a,b). The essential factor in the treatment of metal poisoning is therefore to prevent the accumulation of excess metals which may cause irreversible damage to the biomolecules. In the present study, in vitro experiments have been conducted to examine whether lead has any direct action on some digestive enzymes and the effectiveness of the chelating agent EDTA in reversing the early stages of intoxication.

Materials and Methods

Specimens of Channa punctatus in living condition were collected from the local fresh water sources. After acclimatization for 24 hours, the fishes were dissected and the different parts of the alimentary canal and liver were separated from the adjoining tissues. The tissues were weighed and homogenized in cold 0.25M sucrose solution using a chilled Potter Elvehjem homogenizer. The homogenates were centrifuged for 20 minutes at 2000 g and the clear supernatant fluids adjusted to 10% (W/V) strength were used as the

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source of enzymes. 0.016M sodium β-glycerophosphate was used as the substrate at pH 9.3 for alkaline phosphatase and the enzyme activity was determined according to the method of BODANSKY (1933). Lipase activity was estimated following the method of BIER (1955) with tween 20 as substrate. The activities of peptidases were determined by the method of SMITH (1955). Enzyme protein in the homogenates was estimated by the method of LOWRY et al.(1951) using bovine serum albumin as standard. The test described by FISHER (1950) was employed to calculate the statistical significance between the control and experimental values.

Lead nitrate and EDTA were dissolved in double distilled water so as to form a 5 μM stock solution and further dilutions were made as desired.

Results

The results of the experiments are shown in table 1 to 4. These *in vitro* studies reveal that lead is quite effective in inhibiting the activities of all the enzymes examined here. In comparison to alkaline phosphatase, the digestive enzymes, lipase and peptidases showed more inhibition. Maximum inhibition was observed in the activity of lipase in liver and intestine. The degree of inhibition is directly proportional to the increase in the concentration of lead nitrate in the incubation medium. To test the effectiveness of the chelating agent EDTA in restoring the inhibitory action of lead on enzymes, normal tissue homogenates of different parts of the digestive system were preincubated with lead nitrate and these samples were then incubated with different concentrations of EDTA. The results given in table 5 show that EDTA is capable of restoring the activity of all the enzymes studied here. There is a gradual restoration, and the degree of restoration increased with the increase in the concentration of EDTA.

Discussion

In our earlier studies on the effect of *in vivo* exposure of lead on the digestive system of *Channa punctatus*, it has been observed that the activities of alkaline phosphatase, lipase, aminotripeptidase and glycylglycine dipeptidase are inhibited. This inhibition can adversely effect the processes of digestion and absorption of food material, thereby decreasing the nutritive value of the fishes. The mechanism of action

TABLE 1

In vitro inhibition of liver enzymes by lead nitrate.[a]

Enzymes	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg. Inorg. phosphate/mg. protein/hr.	Control	0.0469 ± 0.0016	
	.4	0.0429 ± 0.0007	8.52(+)b
	.8	0.0406 ± 0.0006	13.43(+)
	1.6	0.0379 ± 0.0018	19.18(+)
Lipase units	Control	57 ± 1.68	
	.4	33 ± 2.07	42.10(+)
	.8	28 ± 1.00	50.87(+)
	1.6	31 ± 1.00	45.60(+)
Aminotripeptidase mg.Glycine/ mg.protein/hr.	Control	0.1840 ± 0.0056	
	.4	0.1700 ± 0.0013	7.60(-)
	.8	0.1340 ± 0.0033	27.17(+)
	1.6	0.1340 ± 0.0033	27.17(+)

TABLE 2

In vitro inhibition of pyloric caeca enzymes by lead nitrate.

Enzymes	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg.Inorg. phosphate/mg. protein/hr.	Control	0.0601 ± 0.0006	
	.4	0.0562 ± 0.0008	6.50(+)
	.8	0.0539 ± 0.0010	10.31(+)
	1.6	0.0536 ± 0.0009	7.06(+)
Aminotripeptidase mg.Glycine/ mg.protein/hr.	Control	0.2220 ± 0.0063	
	.4	0.1940 ± 0.0043	12.61(+)
	.8	0.1630 ± 0.0033	26.57(+)
	1.6	0.1530 ± 0.0033	31.08(+)
Glycylglycine dipeptidase mg.Glycine/ mg.protein/hr.	Control	0.2020 ± 0.0036	
	.4	0.1710 ± 0.0046	15.34(+)
	.8	0.1590 ± 0.0033	21.28(+)
	1.6	0.1490 ± 0.0033	26.23(+)

a. Values are mean ± S.E.
b. (+) indicates statistically significant differences from control values at 95% confidence interval.

TABLE 3

In vitro inhibition of intestinal enzymes by lead nitrate.[a]

Enzymes	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg. Inorg. phosphate/mg. protein/hr.	Control	0.0601 ± 0.0011	
	.4	0.0562 ± 0.0009	6.48(+)b
	.8	0.0539 ± 0.0006	10.31(+)
	1.6	0.0536 ± 0.0064	10.81(+)
Lipase units	Control	48 ± 1.34	
	.4	42 ± 1.45	12.50(+)
	.8	37 ± 1.68	22.91(+)
	1.6	33 ± 1.68	31.25(+)
Aminotripeptidase mg. Glycine/ mg. protein/hr.	Control	0.2310 ± 0.0054	
	.4	0.2190 ± 0.0033	5.19(-)
	.8	0.1960 ± 0.0023	15.15(+)
	1.6	0.1840 ± 0.0050	20.34(+)
Glycylglycine dipeptidase mg. Glycine/ mg. protein/hr.	Control	0.2080 ± 0.0036	
	.4	0.1890 ± 0.0050	9.13(+)
	.8	0.1670 ± 0.0069	19.71(+)
	1.6	0.1590 ± 0.0040	23.55(+)

TABLE 4

In vitro inhibition of stomach enzyme by lead nitrate.

Enzyme	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg. Inorg. phosphate/mg. protein/hr.	Control	0.0449 ± 0.0005	
	.4	0.0438 ± 0.0009	2.45(-)
	.8	0.0429 ± 0.0010	4.45(-)
	1.6	0.0432 ± 0.0009	3.78(-)

a. Values are mean ± S.E.
b. (+) indicates statistically significant differences from control values at 95% confidence interval.

TABLE 5

Restoration of *in vitro* lead nitrate inhibited enzyme activities by EDTA[a].

Enzymes	Concentration uM	Liver	% Restoration	Intestine	% Restoration
Alkaline phosphatase mg. Inorg. phosphate/ mg. protein/ hr.	Control	0.1013 ± 0.0027		0.1280 ± 0.0046	
	Experimental	0.0813 ± 0.0013		0.1187 ± 0.0013	
	Expt.+ EDTA(1)	0.0913 ± 0.0006	50.00	0.1240 ± 0.0023	56.98
	Expt.+ EDTA(2)	0.0947 ± 0.0035	67.00	0.1267 ± 0.0013	86.02
Lipase units	Control	56.7 ± 1.667		47.7 ± 1.334	
	Experimental	31.0 ± 1.000		33.3 ± 1.667	
	Expt.+ EDTA(1)	40.7 ± 0.331	32.29	39.3 ± 0.667	41.60
	Expt.+ EDTA(2)	46.7 ± 0.883	61.08	42.7 ± 0.667	65.27
Aminotripeptidase mg. glycine/ mg. protein/ hr.	Control	0.1843 ± 0.0056		0.2310 ± 0.0055	
	Experimental	0.1337 ± 0.0033		0.1840 ± 0.0050	
	Expt. +EDTA(1)	0.1703 ± 0.0013	72.80	0.1987 ± 0.0023	31.27
	Expt. +EDTA(2)	0.1773 ± 0.0049	86.18	0.2213 ± 0.0052	79.30

a. Values are mean ± S.E.

of heavy metals on physiological processes is not well understood. It has been proposed by PASSOW et al.(1961) that the inhibition in enzyme activities produced by heavy metals may be due to the direct binding of the metal with the enzyme protein or due to the damage of cell organelles. Heavy metals inhibit the activity of enzymes not only *in vivo* but *in vitro* also. *In vitro* inhibition of succinic dehydrogenase by manganese has been reported by SETH & HUSSAIN (1974) and the activity was restored by treatment with chelating agents. In our *in vitro* experiments with mercury (SASTRY & GUPTA, 1974d) and the present experiment with lead, inhibition is observed in the activities of alkaline phosphatase, lipase, aminotripeptidase and glycylglycine dipeptidase. This inhibition is restored to a considerable extent by treatment with the chelating agent EDTA, indicating that the metal is bound to enzyme protein. A number of workers have reported damage of tissues caused by heavy metals (SASTRY & GUPTA, 1977c, CHANDRA & IMAM, 1973, CHANG & HARTMANN, 1972, CHANG & YAMAGUCHI, 1974). The damage caused to cell organelles may result in the decreased synthesis of enzyme protein but very little information is available on this aspect. HIRTH (1964) in his *in vitro* studies has stated that the mechanism of enzyme inhibiting effect revolves mainly around the affinity of mercury and lead to the SH group. JONDERKO (1964) has reported similar inhibition of alkaline phosphatase and dehydrogenase by lead and mercury salts. Further work is required to understand the mechanism of binding of heavy metals with the enzymes and on their effect on protein synthesis.

Summary

The effect of lead on alkaline phosphatase, lipase, aminotripeptidase and glycylglycine dipeptidase in the liver and digestive tract of *Channa punctatus* is investigated *in vitro*. Mercury inhibits the activities of all these enzymes and the degree of inhibition increased with the increase in the concentration of the metal. Addition of EDTA, a chelating agent, restored the mercury inhibited enzyme activity and the degree of restoration was related to the concentration of the chelating agent.

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