Bull. Environm. Contam. Toxicol. 20,729-735 (1978)

In vitro Inhibition of Digestive Enzymes by Heavy Metals and Their Reversal by Chelating Agent: Part I. Mercuric Chloride Intoxication

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Introduction

Industrial wastes cause contamination of world water ways and endanger the life of aquatic fauna, of which fishes are the most senstive group (MATHIS & KEVERN, 1975). Considerable information is available on the residual toxicity and other environmental factors of the heavy metals (BROOKS et al. 1976, CHRISTENSEN & TUCKER, 1976, DAVIS et al. 1973, DUODROFF & KATZ, 1953, MERLINI & POZZI, 1977). In recent years alteration in enzymic activities due to mercury has been proposed by us (SASTRY & GUPTA, 1977a,b) and others (HINTON et al. 1973, ULMER & VALLE, 1969) but little information is available on the mechanism of action of these heavy metals on the physiological functioning of different organ systems. According to PASSOW et al. (1961) the inhibition of enzyme activities by heavy metals is either due to the direct binding of the metal with enzyme protein or the toxic effects produced by them in the tissues. In the present study in vitro experiments have been conducted to examine whether mercury has any direct action on some digestive enzymes and the effectiveness of the chelating agent EDTA in reversing the early stages of inhibition.

Material and Methods

Living specimens of Channa punctatus 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 source of enzymes. 0.016M sodium B-glycerophosphate was used as the substrate at pH 9.3 for alkaline phosphatase and the enzyme activity was determined according to the

0007-4861/78/0020-0729 $01.40

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.

Mercuric chloride 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 tables 1 to 4. These in vitro studies reveal that mercury is quite effective in inhibiting the activities of all the enzymes examined here. Maximum inhibition is observed in the activity of lipase in liver and intestine. The degree of inhibition is directly proportional to the increase in the concentration of mercuric chloride in the incubation medium. To observe the effect of EDTA in restoring the inhibitory action of mercury on enzymes, normal tissue homogenates of different parts of the digestive system were preincubated with mercury 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

Our earlier in vivo studies (SASTRY & GUPTA, 1977a,b) have revealed that mercury inhibits the activities of alkaline phosphatase, aminotripeptidase, glycylglycine dipeptidase and lipase in the digestive system of Channa punctatus. Though it is known that heavy metals inhibit the activity of enzymes, the mechanism of action is not well understood. According to PASSOW et al. (1961) toxic effects of metals results from their binding with biologically active body constituents such as lipids, amino acids and proteins. Further, as heavy metals also produce damage to the tissues (SASTRY & GUPTA, 1977c, CHANDRA & IMAM, 1973), the decrease in the activity of enzymes may be due to a decreased synthesis of enzymes. Very little, if at all any,

TABLE 1

In vitro inhibition of liver enzymes by mercuric chloride.[a]

Enzymes	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg. Inorg. phosphate/mg. protein/hr.	Control	0.0426 ± 0.0012	
	.4	0.0370 ∓ 0.0010	13.14(+)[b]
	.8	0.0366 ∓ 0.0018	14.08(+)
	1.6	0.0354 ∓ 0.0028	16.90(+)
Lipase units	Control	53 ± 3.31	
	.4	38 ∓ 1.68	28.30(+)
	.8	32 ∓ 1.68	39.60(+)
	1.6	29 ∓ 1.68	45.28(+)
Aminotri-peptidase mg. Glycine/ mg. protein/ hr.	Control	0.179 ± 0.0056	
	.4	0.171 ∓ 0.0017	3.35(-)
	.8	0.144 ∓ 0.0033	19.55(+)
	1.6	0.137 ∓ 0.0057	23.46(+)

TABLE 2

In vitro inhibition of pyloric caeca enzymes by mercuric chloride.

Enzymes	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg. Inorg. phosphate/mg. protein/hr.	Control	0.0607 ± 0.0012	
	.4	0.0514 ∓ 0.0009	15.32(+)
	.8	0.0486 ∓ 0.0018	19.93(+)
	1.6	0.0466 ∓ 0.0019	23.22(+)
Aminotri-peptidase mg. Glycine/ mg. protein/hr.	Control	0.2280 ± 0.0060	
	.4	0.1940 ∓ 0.0036	14.91(+)
	.8	0.1880 ∓ 0.0050	17.54(+)
	1.6	0.1530 ∓ 0.0066	32.89(+)
Glycylglycine dipeptidase mg. Glycine/mg. protein/hr.	Control	0.2050 ± 0.0036	
	.4	0.1750 ∓ 0.0046	14.63(+)
	.8	0.1590 ∓ 0.0033	22.43 (+)
	1.6	0.1530 ∓ 0.0033	25.36(+)

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 mercuric chloride.[a]

Enzymes	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg. Inorg. phosphate/mg. protein/hr.	Control	0.0622 ± 0.0011	
	.4	0.0596 ± 0.0007	4.18(-)
	.8	0.0574 ± 0.0009	7.71(+)b
	1.6	0.0587 ± 0.0007	5.62(+)
Lipase units	Control	49 ± 0.91	
	.4	40 ± 0.40	18.36(+)
	.8	33 ± 1.45	32.65(+)
	1.6	35 ± 1.81	28.57(+)
Aminotripeptidase mg. Glycine/ mg. protein/hr.	Control	0.2310 ± 0.0054	
	.4	0.2150 ± 0.0072	6.92(-)
	.8	0.1860 ± 0.0073	19.48(+)
	1.6	0.1710 ± 0.0040	25.90(+)
Glycylglycine dipeptidase mg. Glycine/mg. protein/hr.	Control	0.2120 ± 0.0060	
	.4	0.1960 ± 0.0023	7.54(+)
	.8	0.1800 ± 0.0078	15.09(+)
	1.6	0.1670 ± 0.0069	21.22(+)

TABLE 4

In vitro inhibition of stomach enzyme by mercuric chloride.

Enzyme	Concentration uM	Specific activity	% inhibition
Alkaline phosphatase mg. Inorg. phosphate/mg. protein/hr.	Control	0.0428 ± 0.0012	
	.4	0.0363 ± 0.0009	15.18(+)
	.8	0.0378 ± 0.0006	11.68(+)
	1.6	0.0378 ± 0.0006	11.68(+)

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

TABLE 5

Restoration of *in vitro* mercuric chloride inhibited enzyme activities by EDTA.[a]

Enzymes	Concentration uM	Liver	% Restoration	Intestine	% Restoration
Alkaline phosphatase mg. Inorg. phosphate/ mg. protein/ hr.	Control	0.0947 ± 0.0024		0.1260 ± 0.0030	
	Experimental	0.0787 ± 0.0013		0.1000 ± 0.0023	
	Expt.+ EDTA(1)	0.0853 ± 0.0013	41.25	0.1173 ± 0.0013	66.5
	Expt.+ EDTA(2)	0.0920 ± 0.0020	83.12	0.1233 ± 0.0017	89.6
Lipase units	Control	53.3 ± 3.333		49.3 ± 0.883	
	Experimental	28.7 ± 1.856		34.7 ± 2.603	
	Expt.+ EDTA(1)	38.3 ± 0.883	39.0	39.7 ± 2.603	34.24
	Expt.+ EDTA(2)	45.7 ± 0.331	69.10	43.3 ± 1.763	58.90
Aminotripeptidase mg-glycine/ mg.protein/ hr.	Control	0.1787 ± 0.0056		0.2310 ± 0.0055	
	Experimental	0.1370 ± 0.0058		0.1710 ± 0.0040	
	Expt.+ EDTA(1)	0.1640 ± 0.0040	67.74	0.2023 ± 0.0052	52.16
	Expt.+ EDTA(2)	0.1697 ± 0.0016	78.41	0.2153 ± 0.0033	73.80

a. Values are mean ± S.E.

information is available on the effects of heavy metals on the mechanism of protein synthesis. The present experiments point out that mercury binds with the enzyme proteins and inhibits their activity. This inhibition is reversible as restoration of enzyme activity is observed by treatment with the chelating agent, EDTA. However, further work is needed to understand the mechanisms of binding. The inhibition in the activities of different enzymes observed here may be due to the combined action of mercury on the enzyme protein and damage to the cell organelles. Inhibition in the activities of succinic dehydrogenase by manganese has been reported by SETH & HUSSAIN (1974) who have attributed it to the binding of manganese with the mitochondrial membrane or the enzyme itself. According to PENALVER (1957) and WYNTER (1962) chelating agents are more effective in restoring the physiological changes in early stages of poisoning by metals than in chronic stages, where permanent alterations take place.

Summary

The effect of mercury 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.

Acknowledgement

The authors are thankful to Dr. V.P. Agrawal, Principal of this institution, for his encouragement and support in this work. Financial assistance by University Grants Commission, New Delhi is gratefully acknowledged.

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