Bull. Environ. Contam. Toxicol. (1988) 41:324–328

Bulletin of Environmental Contamination and Toxicology

In vitro Effect of Mercury and Cadmium on Brain Ca^{2+}-ATPase of the Catfish *Ictalurus punctatus*

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Freshwater teleosts possess a calcium activated adenosine triphosphatase (Ca^{2+}-ATPase, EC 3.6.1.3) in the gills (Ma et al 1974; Shephard and Simkiss 1978) and brain (Desaiah and Koch 1975a , 1975b, Verma et al 1983) which has an important role in calcium metabolism. Calcium plays a major role as an important intracellullar second messenger in the central nervous system (Rubin 1978, Wallace and Nairn 1985). Further, calcium has been found to be involved in neurotransmitter release and turnover, generation of Ca^{2+} spikes and regulation of Ca^{2+}-dependent K^{+}-channels (Delorenzo 1985; Wallace and Nairn 1985). Earlier studies have demonstrated that pesticides are found to inhibit the Ca^{2+}-ATPase activity in the gills of fish (Desaiah and Koch 1975a, 1975b; Thebault and Decaris 1983; Bansal et al 1985). Although heavy metals have been shown to affect Na^{+}-K^{+}-ATPase in the brain (Verma et al 1983) and Ca^{2+}-ATPase in the gills (Shephard and Simkiss 1978; Bansal et al 1985) of fish, the mode of action of heavy metals on fish brain Ca^{2+}-ATPase has not been clearly understood to date. Since heavy metals are known to produce neurotoxic action in higher vertebrates (Bouquegneau 1977; Rajanna et al 1983), the disruptive action of heavy metals on 'calcium pump' in fish brain could be explained if they were found to affect this enzyme. Hence, this study was undertaken to evaluate the *in vitro* effect of divalent metals, mercury and cadmium on Ca^{2+}-ATPase in the brain of catfish, *Ictalurus punctatus*.

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MATERIAL AND METHODS

Pond cultured channel catfish, Ictalurus punctatus were obtained from the catfish farm at Brandon, Mississippi. After acclimatization, each fish weighing 9.61 $\pm$ 0.53 g was decapitated just anterior to the opercula, brain tissue was excised and placed in ice-cold homogenizing medium (0.32 M sucrose and 10 mM imidazole pH 7.5). The tissues were homogenized and centrifuged at 750 x g for 15 min. The supernatant was again centrifuged at 9000 x g for 15 min. The resulting supernatant was centrifuged at 12000 x g for 30 min. The pellet comprising nerve ending particles was suspended in homogenizing medium, quick frozen and stored for a week or less at -80°C.

Mercuric chloride (99% pure) and cadmium chloride (99% pure) obtained from Sigma Chemical Co. were used. Stock solutions of heavy metals were prepared in ethanol and appropriate dilutions were made to obtain the required concentrations of the heavy metal. Ethanol was used for better dispersion into the protein. 1.5 µL of test solutions were added to obtain the final concentration. Ethanol at the concentration used in the study had no measurable effect on ATPase activity (see Mehrotra et al 1985).

Ca^{2+}, Mg^{2+}-ATPase activity was measured by determining the inorganic phosphate liberated from the hydrolysis of ATP. The reaction medium contained 135 mM imidazole (pH 7.5), 5 mM $MgCl_2$, 4 mM ATP and 30 to 40 µg enzyme protein. The reaction was incubated at 37°C for 30 min and stopped by the addition of 0.1 mL of 50% TCA. The inorganic phosphate liberated was then estimated by the method of Lowry and Lopez (1946). Total ATPase activity was measured with Ca^{2+} and Mg^{2+} in the reaction mixture, while Mg^{2+}-ATPase activity was measured in the presence of 0.5 mM EGTA. Ca^{2+}-ATPase activity was obtained by subtracting Mg^{2+}-ATPase activity from total ATPase activity. The enzyme activity was expressed as µM Pi/mg protein/h. Protein was determined by the method of Lowry et al (1951), using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Table 1 details the Ca^{2+}-ATPase activity in the synaptosomal fraction of catfish brain after in vitro treatment with various concentrations of mercury and cadmium.

Table 1. In vitro effect of mercury and cadmium on brain Ca^{2+}-ATPase in Ictalurus punctatus.

Concentration of metal (μM)	$HgCl_2$ Specific activity ± SD	$HgCl_2$ % Inhibition	$CdCl_2$ Specific activity ± SD	$CdCl_2$ % Inhibition
0.0	11.26 ± 0.74	-	12.48 ± 0.87	-
0.1	9.39 ± 0.36*	16.60	12.19 ± 1.36	2.3
0.3	8.72 ± 0.41*	22.55	11.99 ± 0.20*	3.9
0.5	6.80 ± 0.73*	39.61	11.88 ± 1.14	4.8
1.0	6.19 ± 0.20*	45.03	11.02 ± 0.08*	11.7
3.0	6.00 ± 0.06*	46.71	8.52 ± 0.08*	31.7
5.0	4.95 ± 0.23*	56.03	8.27 ± 0.36*	36.3
10.0	3.89 ± 0.16*	65.45	7.13 ± 1.69*	42.9
20.0	3.89 ± 0.23*	65.45	6.52 ± 0.43*	47.8
30.0	3.76 ± 0.25*	66.61	5.85 ± 0.33*	53.1
50.0	3.33 ± 0.25*	70.43	4.97 ± 0.66*	60.2

*Values significantly different from control at $P < 0.05$.

Both the divalent cations inhibited the Ca^{2+}-ATPase activity in brain. Inhibition of enzyme activity by mercury was observed at concentration as low as 0.1 μM, while similar effect by cadmium was recorded at 1 μM. Concentration dependent inhibition by $HgCl_2$ was evident below 10 μM level. When percent inhibition was calculated (Table 1), the values significantly ($P < 0.05$) different from control occurred at 0.1 and 3.0 μM concentration of mercury and cadmium respectively. The IC_{50} level of mercury obtained through dose-response curve was 3.8 μM, while that of cadmium was 22.0 μM respectively.

The present work confirms the presence of Ca^{2+}-activated ATPase in the brain of freshwater catfish Ictalurus punctatus. The enzyme is similar to that described by Bansal et al (1985) in another catfish Saccobranchus fossilis. The results reported here show that the two metalic salts can cause a considerable reduction in the activity of Ca^{2+}-ATPase or 'calcium pump' activity of catfish brain. Similar experiments carried out on the enzyme from the gill of Saccobranchus fossilis indicate comparable inhibition by the two metals (Bansal et al 1985). Further, the IC_{50} level of mercury for the brain Ca^{2+}-ATPase of Ictalurus punctatus is significantly different from that of cadmium for the same species but were similar to that reported for the gill and heart of Saccobranchus fossilis (Bansal et al 1985). Mercury was also found to inhibit the Na^+-K^+-ATPase in the brain of the fishes Ophicephalus punctatus (Sastry and Sharma 1980) and Notopterus notopterus (Verma et al 1983). These findings suggest that, this inhibitory activity is a major factor in the ability of these metals to disrupt calcium

metabolism in the different tissues. It could be conceived that the two divalent cations may alter cellular configuration by interferring with Ca^{2+}-sites on Ca^{2+}-ATPase. Since ATP is an integral part of the membrane, the activity of the enzyme would be altered thus disrupting the synaptic transmission.

Acknowledgement. The authors express their appreciation to Margaret Collins for typing the manuscript.

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Received September 24, 1987; accepted April 20, 1988.