Bull. Environ. Contam. Toxicol. (1988) 40:759–763

Environmental Contamination and Toxicology

Cadmium Induced Inhibition of Na^+/K^+ ATPase Activity in Tissues of Crab *Scylla serrata* (Forskal)

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Heavy metals discharged from industries these days are a major source of pollution which has become threat to all forms of life. A measure of metabolism may be a most sensitive parameter since it integrates many factors such as enzyme activity (Dhavale and Masurekar 1986a), biochemical contents (Dhavale and Masurekar 1986b) and physiological response. The ability to accurately characterize enzymes with respect to their distribution and kinetics makes them attractive indices of stress. Adenosine triphosphatases (ATPases) which are involved in osmoregulation and intracellular functions, e.g. the 'sodium pump', are very sensitive indicators of trace metal toxicity (Haya and Waiwood 1983). Na^+/K^+-ATPase (EC 3.6.1.3) are involved in physiological processes and the inhibition by xenobiotics may produce significant toxic effect to the cells. Euryhaline organisms live in an environment where they are subjected to salinity variations due to tidal fluctuations. Adaptation to these changes is critical to their survival and is reflected in a variety of osmoregulatory functions which are mediated by Na^+/K^+-ATPase. The concept of Na^+/K^+-APTase being intimately involved in active transport of ions across biological membranes has gained wide acceptance in recent years. Interference with osmoregulation may restrict the animial's ability to adapt to salinity changes. Several studies have shown that crustaceans are highly sensitive to metal pollutants, particularly when acclimated to low salinities (Jones 1975). The present study was initiated to compare the characteristics of Na^+/K^+-ATPase activity in hepatopancreas and gills of crabs Scylla serrata (Forskal) exposed to acute and sublethal concentrations of cadmium chloride for defined periods with those from undosed animals.

MATERIALS AND METHODS

Juvenile crabs of intermoult stage and more or less of uniform size (4.5 to 5.5 cm across) and average weight (24.75 gm) were collected from Bassein Creek near Bombay. The crabs

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were acclimated for 7 days to the laboratory conditions in glass aquaria (92x46x46 cm) containing sea water, devoid of contaminants, which had temperature 25-27°C, pH 7.8-8, dissolved oxygen 6.3 to 6.8 mg/L and salinity 30‰. Identical conditions were maintained during experimentation. Stock solutions were prepared by dissolving cadmium chloride ($CdCl_2$. 2.5 H_2O) in distilled water. The crabs were exposed to acute concentration of cadmium (24.00 mg/L) for 4 days and sublethal concentrations (0.3, 0.6 and 1.5 mg/L) for 10, 20 and 30 days each in glass aquaria, each containing 10 crabs and 3 L of well-aerated sea water (with similar water quality as mentioned above) collected at the same site of catchment area of crabs. A separate control was maintained for each exposure period. After feeding the crabs with frozen clam flesh, the water in the tank was renewed daily and a predetermined quantity of cadmium was added to the water of experimental aquariums. Both experimental and control animals received natural 12 h photoperiod and 27°C temperature. Following exposure of animals to different sublethal concentrations, specimens from experimental and control groups were collected at 10-day intervals. The specimens were autopsied and heptatopancreas and gills were collected immediately. They were weighed on single pan electric balance. A known amount of hepatopancreas and gill (pooled from two specimens) were stored separately in the deep freezer at the temperature of -20°C for determining Na^+/K^+-ATPase activity by using the techniques described by Bonting et al (1961). For enzyme assay the frozen tissue was homogenized with a tissue grinder in 3-4 mL of the apppropriate assay medium without ATP (ATP was added after homogenization). Aliquots of 40 µL tissue homogenate were placed in microcentrifuge tubes and centrifuged (3000 g for 10 min). The remaining tissue homogenate was frozen and later analyzed for protein by the method of Lowry et al (1951). Sample control remained chilled in ice and each received 200 µL of 10% TCA after 1 h. A 150 - µL aliquot of each sample was transferred to a separate beaker and analyzed for inorganic phosphate (Fiske and SubbaRow 1925). The resulting color was read within 1 h at 660 mµ with Spectronic 20 spectrophotometer. Results are expressed as µmol of inorganic phosphate released per mg of tissue protein per h incubation at 37°C. The statistical significance between the treated and control groups was calculated by Student's 't' test (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The effects of different concentrations of cadmium exposure in the gill and hepatopancreas are presented in Table 1. The activity of this enzyme was found to be inhibited in general in both the tissues, except some initial stimulations (ranging from 10 to 16%) at sublethal concentration of 0.3

mg/L. Though inhibition was more pronounced in gill than hepatopancreas, the pattern was very much similar with significant ($P < 0.05$) inhibition at 10 and 20 days of exposure at 1.5 mg/L concentrations.

ATPase is located in the cell membrane and has been implicated in the active transport of Na^+ and K^+ across cell membrane. Metals may alter the enzyme activity or function in several ways. They can bind to a number of sites on proteins including imidazole, histidyl, carboxyl and especially sulphydryl side chains (Verma et al 1983). Metals may also bring about changes in concentrations of cofactors or reactants by altering membrane permeability, including that of mitochondria, again indirectly affecting enzyme activity. Similar inhibition in the enzyme activity was observed in gills of rock crab exposed to cadmium (Tucker and Mattle 1980) and Zn exposed lobsters (Haya and Waiwood 1983). Since ATPase is an integral part of the membrane, the activity of enzyme would be altered and movement of substances by active transport would be blocked, thus disrupting the functions of the organs (gills in this case) where ATPase has been inhibited. The general body wall of the crab is relatively impermeable; the gills serve as a major organ for osmotic regulation as well as for respiration. Heavy metals inhibit Na^+/K^+-ATPase activity and interfere with osmoregulatory mechanisms of aquatic organisms. Na^+/K^+-ATPase has been shown to play an important role in maintaining hemolymph ion concentrations different from ion concentrations of their environment (Quinn and Lane 1966) and cause elevation of electrolytes in the blood which denotes acute stress response (Larsson et al 1981). Similar elevation in osmotic and ionic regulation was observed in Cancer magister and Hemigraphus nudus (Caldwell 1974), and cadmium exposed crab, Scylla serrata (Dhavale 1984). The inhibition of enzyme activity in hepatopancreas is due to cadmimum toxicity which is known to have direct adverse effect on mitochondria, and it is therefore likely that the metal affects the metabolism of the hepatic cells resulting in the inhibition of enzyme activity there. During long term exposure, cadmium is bound to a metallothionein-like protein at the site of absorption and then transferred to storage organ like hepatopancreas as observed by Olafson et al (1979) in Scylla serrata. It appears that inhibition of Na^+/K^+-ATPase activity in the crabs exposed to cadmium is correlated with alterations in the ionic concentration of blood and the damage caused to the tissues like gills and hepatopancreas.

In summary, the results reported in this paper indicate that inhibition of Na^+/K^+-ATPase activity may occur in tissues of crabs following exposure to cadmium. Inhibition of this ion transport enzyme which is intimately coupled to osmoregulation may partially explain the toxicity of cadmium to crabs.

Table 1 Effect of different concentrations of cadmium on tissue Na^+/K^+-ATPase activity[a] in crab Scylla serrata (Values are mean of 5 determinations ± S.E.M.)

Period Days	Control	Cadmium concentrations (mg/L)			
		0.3	0.6	1.5	24.0
GILL					
4	0.855 ±0.124				0.321** ±0.061 (-62.45)
10	0.886 ±0.167	0.983 ±0.212 (+10.94)[b]	0.474* ±0.110 (-46.5)	0.398** ±0.141 (-55.07)	
20	0.997 ±0.216	0.736 ±0.436 (-26.17)	0.373** ±0.094 (-62.58)	0.289** ±0.135 (-71.01)	
30	0.798 ±0.138	0.595 ±0.234 (-25.63)	0.557 ±0.136 (-30.20)	0.441* ±0.197 (-44.73)	
HEPATOPANCREAS					
4	0.265 ±0.028				0.168 ±0.089 (-36.6)
10	0.273 ±0.031	0.278 ±0.041 (+1.83)	0.187 ±0.0214 (-31.50)	0.112* ±0.043 (-58.9)	
20	0.245 ±0.051	0.286 ±0.038 (+16.73)	0.193 ±0.024 (-21.2)	0.093* ±0.031 (-62.0)	
30	0.282 ±0.044	0.243 ±0.108 (-13.8)	0.227 ±0.183 (-19.5)	0.202 ±0.196 (-28.36)	

a Activity is expressed in µmol Pi/mg protein/h at 37°C
b Values in parenthesis indicate % inhibition (-) or stimulation (+).
** $P < 0.01$
* $P < 0.05$ (Significant when compared with control values)

Acknowledgments. One of us (DMD) is thankful to the University Grants Commission of India for financial assistance in the form of Teacher Fellowship. Thanks are also due to Shri MG. Dhavale for his constant help.

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Received July 24, 1987; accepted November 25, 1987.