Bull. Environ. Contam. Toxicol. (1995) 55:723–729

Environmental Contamination and Toxicology

Effects of Cr (VI) on ATPases in the Brain and Muscle of Mudskipper, *Boleophthalmus dentatus*

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Received: 3 February 1994/Accepted: 20 March 1995

Sublethal effects of chromium on fish studied under laboratory conditions appeared to be directly related to inhibition of metabolic processes (Nath and Kumar 1987). ATPases are responsible for movement of ions through the membrane and thereby regulate, among others, osmotic pressure, cell volume and thus the membrane integrity. Effects of mercury on the gill and intestine ATPases of mudskipper were thoroughly investigated earlier (Lakshmi et al. 1990 a,b). Patel and Saxena (1983) studied the toxic effects of Cr compounds on different parameters in fish tissues. Effects of Cr (VI) were studied on rainbow trout and roach with special emphasis on ATPases (Kuhnert 1976).

The experimental animal of the present investigations, mudskipper, Boleophthalmus dentatus occurs abundantly on the coastal mudflats. It is an important source in the coastal animal food chain and also occasionaly consumed by the local people. The present communication therefore, deals with the effect of potassium dichromate on five ion dependent ATPases in brain and axial muscular tissues of this coastal teleost.

MATERIALS AND METHODS

The coastal fish species, Boleophthalmus dentatus (Family: Gobidae), commonly known as mudskipper, of about 11.5-12.5 cm in length, were obtained from the coastal mudflats of the Gulf of Katch (India). Animals were acclimated to laboratory conditions for about a week in large cement troughs containing coastal mud and normal sea water. They were maintained at 26oC temperature and were fed ad libitum with chopped prawns. The LC_{50} (96 hr) for potassium dichromate ($K_2Cr_2O_7$) in this species was reported to be 85 mg/L (Lakshmi et al. 1990a). The Cr(VI) stress was imposed by dissolving

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analytical grade of $K_2Cr_2O_7$ (BDH,India) in normal seawater (salinity around 35.4‰) to a desired stock concentration. Four sublethal treatment concentrations (30, 40, 50 & 60 mg/L) were prepared with seawater using the stock Cr solution. At least 10 mudskippers were put in separate glass tanks containing these four Cr concentrations and each treatment exposed for three different time durations (1,2 & 3 d). Separate control groups were maintained along with each treatment. During this time the fish were not fed but were maintained as during acclimation. The experimental media were renewed every 12 hr. Visual observation was carried out on every 2 hr to detect changes in the behavioral activity of the animals in different concentrations for different durations. On the sheduled days (2,3 & 4d), animals were sacrificed and the whole brain was dissected out and placed in chilled buffered sucrose solution. Similarly, muscular tissue was dissected from the middle of the trunk on the lateral line after peeling out the skin and placed in chilled buffered sucrose solution. The whole operation was done within 3 min after the death of the animal. Tissue from all animals exposed for a particular concentration and duration was pooled and 200 mg of each tissue was taken for tissue preparation and enzyme assay as described earlier (Lakshmi et al. 1990a). The activities of five ion dependent ATPases, viz., Na^+K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase, Ca^{2+},HCO_3-ATPase and Mg^{2+},HCO_3-ATPase, were estimated (Lakshmi et al. 1990a). All experiments were repeated at least thrice. A two level nested ANOVA was performed to estimate the statistical significance of treated animals over the controls, from which the Least Significant Difference (LSD) was computed (Sokal and Rohlf 1969).

RESULTS AND DISCUSSION

Visual observations during the experiments showed notable behavioral changes. Animal showed aggressive behaviour with rapid swimming activity soon after exposure to the toxic media. This erratic swimming persisted for sometime and animals became inactive with increasing Cr(VI) concentration and exposure duration. In longer exposure durations in almost all the concentrations fish became stiff and yellowish spots developed on the skin. In higher concentrations and longer exposure durations fish showed paralytic symptoms. Results of the assay of five ion dependent ATPases in the control animals revealed that these enzymes are active in the brain as well as muscular tissues, but their specific activities are tissue specific (Table 1).

Figures 1 & 2 show a significant inhibition in the specific activities of all five ATPases estimated as compared to their respective controls on exposure to

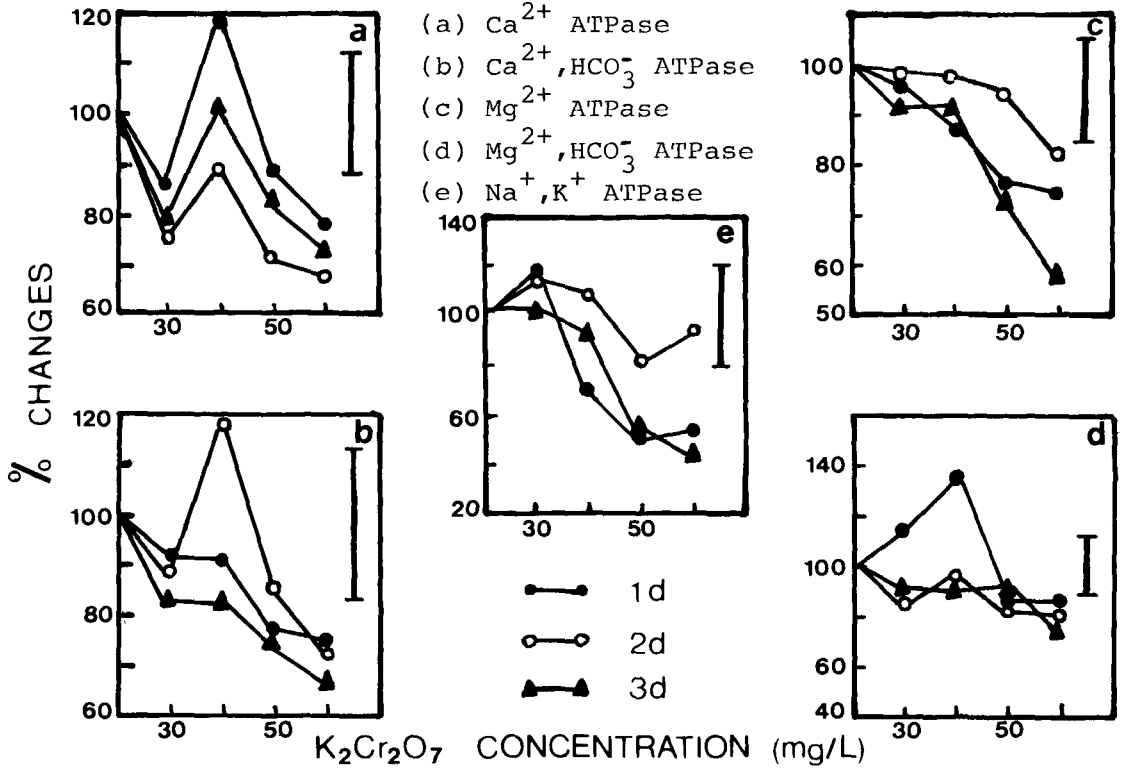

Figure 1. Specific activity of five ion dependant ATPases in the brain of B.dentatus exposed to different concentrations of Cr(VI) for different durations. Values are expressed as % of their respective control. Vertical bars denote the range of LSD at P<5%.

Table 1. Specific activities (uM Pi/mg protein/hr) of different ATPases in the brain and muscle of control animals. Values are expressed as mean ± SE.

Enzyme	Tissue	Specific activity 1 d	2 d	3 d
Ca^{2+}ATPase	Brain	9.23±1.4	11.25±1.7	6.91±1.1
	Muscle	3.76±1.0	7.13±1.0	4.96±0.3
Mg^{2+}ATPase	Brain	10.49±1.4	10.66±1.9	12.67±1.3
	Muscle	6.33±1.4	3.21±0.6	5.16±0.3
Ca^{2+},HCO_3^--ATPase	Brain	9.19±1.0	11.79±1.1	9.00±1.2
	Muscle	6.20±1.3	5.59±0.5	5.23±0.2
Mg^{2+},HCO_3^--ATPase	Brain	10.58±0.8	12.51±2.1	10.47±1.5
	Muscle	5.81±1.2	5.03±0.8	4.57±0.4
Na^+,K^+ATPase	Brain	10.02±0.7	10.68±0.5	8.84±0.3
	Muscle	5.63±0.2	5.54±0.6	5.76±0.5

Cr(VI) stress. In general a significant dose and duration dependent inhibition of Na^+,K^+-ATPase was observed both in brain and muscle. However, non - significant stimulation in the specific activity of this enzyme was observed in 30 mg/L concentration exposed for 1 d. In brain, the Ca^{2+} and Mg^{2+} dependent ATPases showed inhibition (Fig 1). However, in 40 mg/L concentration, a significant stimulation in the specific activity of these enzymes was observed. Specific activities of Ca^{2+},HCO_3^-- and Mg^{2+},HCO_3^-- dependent ATPases showed initial stimulation in 1-d exposure. In muscle, progressive inhibition of the specific activity of all enzymes was observed (Fig 2). In lower concentrations (30 and 40 mg/L) occasional stimulation in the activity of some enzymes was observed.

Results of the present investigations show a general trend of progressive inhibition in the specific activity of the enzyme studied with increasing dose and duration of Cr(VI). The notable inhibition of Na^+,K^+-ATPase could cause disruption in the membrane structure, either in the plasma membrane and/or that of mitochondria resulting in metabolic depression in the animal itself (Jernelov et al. 1978). The ATPase system is responsible for transport of different ions across membrane in the cell (Skou 1975), significantly assists the mechanisms of bioelectrical phenomena, salt and water homeostasis, transport of non-electrolytes, and is reported to be inhibited by different metal ions (Nechay and Saunders 1984). Therefore, the inhibition of Na^+, K^+ATPase

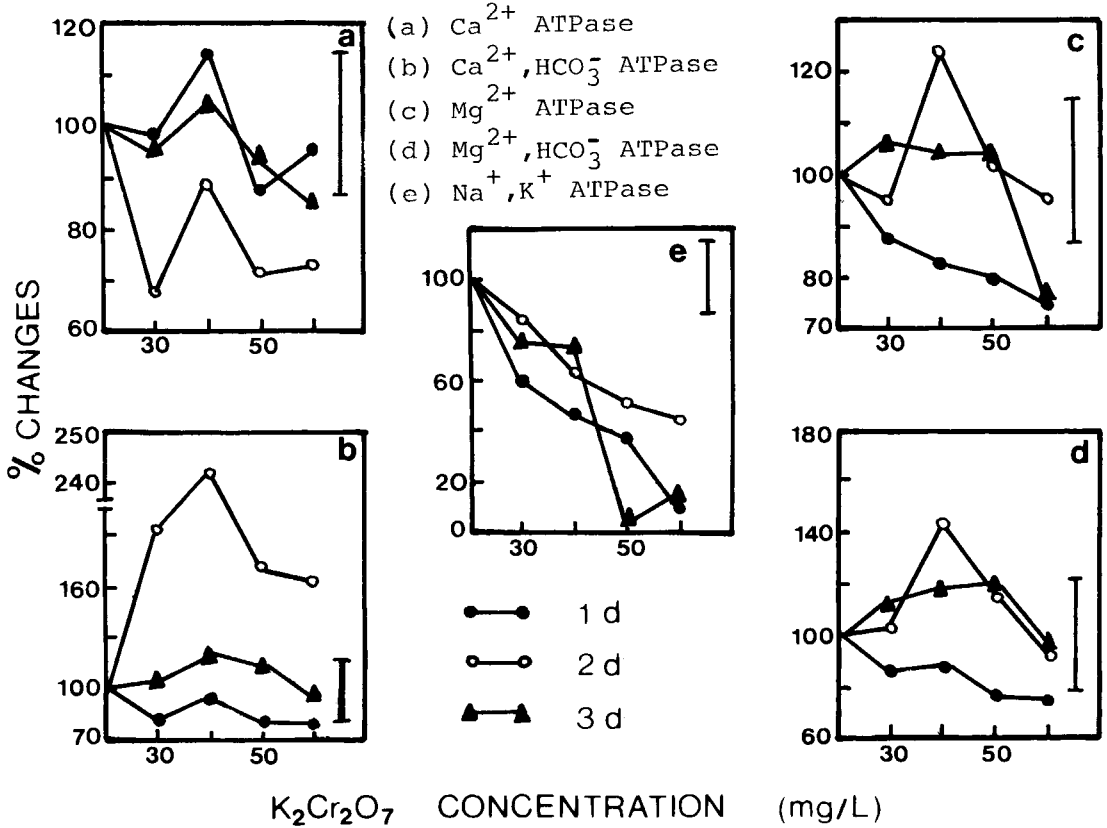

Figure 2. Specific activity of five ion dependant ATPases in the muscle of B. dentatus. Other details are same as Figure 1.

observed in the present study may be due to the formation of bonds between Cr(VI) and available oxide and sulfhydryl groups (Friedman et al. 1987) resulting in a disturbance in ion transport. Heavy metals in their first interaction with the cell are likely to attack the cation pump in the cell membrane. The pumping may also be inhibited by molecules interfering with the production of ATP. The inhibition of Na^+,K^+ pump may cause an uncontrollable entry of Na+ into the cell along the concentration gradient and water molecules follow along the osmotic gradient. This process may cause swelling of the cell and finally membrane ruptures (Jernelov et al. 1978).

Molecules approach the brain only via blood. Reports on toxicological studies on fish brain are scant; hence, the pattern known in mammalian brain intoxicated by various heavy metals are our major source of interpretation. From the present investigation it is concluded that the Cr compound crossed the blood-brain barrier (Syversen 1981) affecting these membrane bound enzymes causing severe neurological impairments. Though, the exact neurotoxicity is not clearly understood at this stage, but like other heavy metals it may cause severe damage to the central nervous system which is evident from the behavioural observations.

On the other hand, molecules reach the muscular tissue of fish via blood as well as by diffusion through skin. Results of the present investigation show that the imposed Cr(VI) stress affects the activity of membrane ATPase system, especially Ca^{2+}, Mg^{2+} as well as Na^+,K^+-ATPases, blocking the normal distribution of those essential ions into the muscular cells. This caused a severe effect on the normal functioning of the muscle. The behavioural observation showed impaired swimming and often paralysis and was perhaps correlated with hampered transport of these ions. Similar observations were reported by Suzuki (1980) using other heavy metals. It is noteworthy that in some cases in the present investigation, stimulation of enzyme activity was observed. This might have resulted from an increased enzyme synthesis by the fish to cope with the increasing osmotic stress (Nimura et al. 1987).

Acknowledgments Financial assistance provided by UGC (India) to RK and CSIR (India) to RL is thankfully acknowledged.

REFERENCES

Friedman J, Fiorela S, Leonard SL, Meir D (1987) Chromium chloride induced chromosomal abberations in human lymphocytes via indirect action. Mutat Res 191:207-210

Jernelov A, Beijer K, Soderland L (1978) General aspects of toxicology. In: Butler GC (ed) Principles of Ecotoxicology, John Wiley & Sons, New York, p 151

Kuhnert PM (1976) Effect of in vivo chromium exposure on Na^{+},K^{+}-ATPase and Mg^{++}-ATPase in several tissues of rainbow trout (Salmo gairdneri). Bull Environ Contam Toxicol 15:383-389

Lakshmi R, Kundu R, Mansuri AP (1990a) Toxicity of mercury to mudskipper, Boleophthalmus dentatus (Cuv & Val) I. Changes in the activity of ATPases in the gills. Acta Hydrochim Hydrobiol 18:581-587

Lakshmi R, Kundu R, Thomas E, Mansuri AP (1990b) Mercuric chloride induced inhibition of different ATPases in the intestine of mudskupper, Boleophthalmus dentatus. Ecotoxicol Environ Saf 21:18-24

Nath K, Kumar N (1987) Efect of hexavalent chromium on carbohydrate metabolism of a freshwater tropical teleost Colisa fasciatus. Bull Inst Zool Acad Sin (Taipei) 26:245-248

Nechay BR, Saunders JP (1984) Inhibition of adenosine triphosphatases in vitro by silver nitrate, silver sulfadiazine. J Environ Pathol Toxicol Oncol 5:119-126

Nimura E, Miura K, Shinobu LA, Imura N (1987). Enhancement of calcium sensitive myosin ATPase activity by cadmium. Ecotoxicol Environ Saf 14:184-189

Patel R, Saxena AB (1983) Effects of potassium chromate on freshwater fishes, Punitus ticto and Channa striatus. Ind J Zool 11:43-49

Skou JC (1975) The $(Na^{+}K^{+})$ activated enzyme system and its relationship to transport of sodium and potassium. Quart Rev Biophys 7:401-434

Suzuki S (1980) Properties of mitochondrial Mg^{++},HCO_3^--stimulated and SCN inhibited ATPase in rat liver, kidney and small intestinal mucosa and some relationship between ATPase and supernatent carbonic anhydrase. Comp Biochem Physiol 670:277-288

Syversen TLM (1981) Distribution of mercury in enzymatically characterized subcellular fractions from developing rat brain after injection of methylmercury and dimethylmercury. Biochem Pharmacol 23:2999-3007