The Effect of *In Vivo* Chromium Exposure on Na/K- and Mg-ATPase Activity in Several Tissues of the Rainbow Trout (*Salmo Gairdneri*)

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ABSTRACT

The effect of in vivo chromium exposure on Na/K- and Mg-ATPase activity was studied in several tissues of the rainbow trout, Salmo gairdneri. Those tissues studied were: intestine, gill, liver, and kidney. Tissue chromium levels were determined for control rainbow trout and trout exposed to 2.5 mg Cr/l (as chromate) for 48 hours. After exposure to chromium, inhibition of Na/K-ATPase activity, but not Mg ATPase activity, was observed. These results may partially explain the detrimental effects of hexavalent chromium on fish.

INTRODUCTION

Many fish tissue Na/K-ATPase[a] studies have been focused on the relationship between Na/K-ATPase activity and osmotic regulation. Most of these investigations have shown that the level of Na/K-ATPase activity parallels the level of active sodium transport in those teleost organs having major osmoregulatory roles, i.e. gill and intestine in marine forms and gill and kidney in fresh-water forms. For example, intestinal mucosal Na/K-ATPase activity and active sodium absorption both appear to be elevated in many teleosts during osmoregulatory adaptation to salt water (OIDE 1967; JAMPOL and EPSTEIN 1970).

In view of the apparent relationship between Na/K-ATPase activity and the osmoregulatory function of certain fish organs, it is of interest to investigate the effect of known toxic environmental pollutants on fish Na/K-ATPase activity. Several studies have been performed concerning the effect of polychlorinated biphenyls on this enzyme in fish: DDT was found to inhibit Na/K-ATPase activity in intestinal tissue from sea water adapted eels (JANICKI and KINTER 1971) and to inhibit kidney and gill Na/K-ATPase of rainbow trout (DAVIS and WEDEMEYER 1971). Also, KINTER et al. and CUTKOMP et al. (1972) have demonstrated that aroclor and DDT adversely affect Mg-[b] and Na/K-ATPase activity in fish. Recently, SCHMIDT-NIELSEN (1974) reported

a. Sodium/potassium activated adenosine triphosphatase.
b. Magnesium activated adenosine triphosphatase.

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that mercuric compounds inhibit Na/K-ATPase activity and interfere with the osmoregulatory mechanisms of aquatic organisms. Studies similar to these concerning other toxic substances may provide important clues to their pathogenic mechanisms.

The present investigation was designed to determine if Na/K- and Mg-ATPase activities (Mg-ATPase is found in conjunction with Na/K-ATPase) in the rainbow trout organs involved in osmoregulation and excretion (intestine, gill, kidney, and liver) are affected by exposure to hexavalent chromium *in vivo*. The toxicity of hexavalent chromium to fish is well established, but little is known about the physiological effects of sub-lethal chromium levels. Heavy metals are usually poisonous to fish externally where they bind to the gill epithelium and interfere with respiration. Hexavalent chromium, however, readily passes through the gill membrane and accumulates in various tissues and organs. The facile absorption of anionic chromium and the lack of any demonstrable gill damage in exposed fish suggests that the hexavalent metal elicits its toxic effect at some internal site.

METHODS

Animals

Two-year old trout were obtained from a commercial supplier (Fisherman's Corral, Burton, Ohio). They were maintained in two 60 gallon wooden holding tanks with separate filtering systems designed to remove particulate matter mechanically and to remove ammonia by bacterial deammonification. A walk-in constant temperature chamber was used to maintain water temperature at 14 ± 1°C under continuous illumination. The fish were fed commercial pellets *ad libitum* once on alternate days.

A 20-gallon glass aquarium with an undergravel bacterial filtering system was used to expose individual trout to 2.5 mg/l hexavalent chromium. The aquarium was kept in the same illuminated constant temperature chamber as the wooden tanks. Prior to chromium exposure each fish was preadapted for 2 days in a separate 20 gallon glass aquarium and then transferred to and maintained in the experimental aquarium containing chromium for 48 hours. Fish were not fed during the experimental period.

Preparation of Tissue

Trout were immobilized by a blow to the head and portions of large intestine, gill, liver and kidney were removed and placed in chilled trout saline solution

(STOKES and FROMM 1964). Small slices of tissue were blotted and weighed to four decimal places. Tissue weights falling within the predetermined weight range (15-20 mg, 30-40 mg, 15-20 mg, 6-8 mg for intestine, gill, liver, and kidney, respectively) necessary for measurable enzyme activity were recorded and quickly frozen in Pyrex glass vials. The tissue was stored at -30°C until analyzed for ATPase activity.

Assay of ATPase Activity

Na/K-and Mg-ATPase activity were determined using techniques described by BONTING et al. (1961). For enzyme assay, the frozen tissue was homogenized with a tissue grinder in 3-4 milliliters of the appropriate assay medium without ATP (ATP was added after homogenization). The tissue homogenate concentrations in mg tissue wet wt/ml were: intestine - 5, gill - 10, liver - 5, and kidney - 2. Six 40 µl-aliquots of tissue homogenate were placed in microcentrifuge tubes. The remaining tissue homogenate was frozen and later analyzed for protein by the method of LOWRY et al. (1951) using bovine serum albumin as a standard. Three sample controls remained chilled in ice and each received 200 µl of 10% TCA after one hour. All six samples were then centrifuged (2,500 rpm) for 10 minutes. A 160 µl-aliquot of each sample was transferred to a separate 2-ml beaker and analyzed for inorganic phosphate (FISKE and SUBBAROW 1925). The resulting color was read within one hour at 660 mµ with a Spectronic 20 spectrophotometer. Results are expressed as micromoles of inorganic phosphate released per milligram of tissue protein per hour incubation at 37°C.

Assay of Tissue Chromium

Atomic absorption spectrophotometry was used to determine tissue chromium. Prior to analysis, digestion of the tissues was accomplished by modifying the techniques reported by DAVIS and GROSSMAN (1971) and FELDMAN et al. (1967). Fish tissues (0.6-6 g) were predigested for one hour in Kjeldahl flasks at room temperature with 3 ml of concentrated nitric acid and 4.5 ml of an acid digestion mixture (70% perchloric and 18 M sulfuric acids; 2:1 v/v). Heat was slowly added until brown nitric acid fumes were given off. The samples were then boiled until after the appearance of white sulfur trioxide fumes when the solution turned bright yellow. After cooling, trivalent chromium was oxidized to hexavalent chromium with potassium permanganate. The hexavalent chromium was then extracted with methyl isobutyl ketone and aspirated into the flame of a Perkin Elmer 305 atomic absorption spectrophotometer. Standards were run in the same manner.

RESULTS

The effect of 48-hour chromium exposure on ATPase activity is shown in Table 1 and Figure 1. Na/K-ATPase activity decreased in all chromium exposed tissue except gill. The kidney was the only chromium exposed tissue that showed a statistically significant decrease in Na/K-ATPase activity. A 50% reduction of intestinal Na/K-ATPase was observed, but it was not statistically significant. In the gill and in the intestine, the changes noted were not significant due to the large variation between samples relative to the low mean Na/K-ATPase activity.

TABLE I

Effect of exposing rainbow trout to 2.5 mg Cr/l for 2 days on tissue Na/K- and Mg-ATPase activity.[a]

ATPase	Tissue	Controls	Chromium Exposed	Level of Significance	% Inhib. or stimulation
Na/K-ATPase					
	Kidney	0.641±0.123	0.234±0.103	0.05	-62.1
	Intestine	0.186±0.084	0.093±0.033	NS[b]	-49.9
	Liver	0.062±0.030	0.059±0.021	NS	- 4.6
	Gill	0.006±0.020	0.022±0.017	NS	*[c]
Mg-ATPase					
	Kidney	0.991±0.164	1.021±0.237	NS	+ 3.1
	Intestine	0.648±0.054	0.627±0.081	NS	- 3.2
	Liver	0.539±0.016	0.542±0.043	NS	+ 0.6
	Gill	0.424±0.038	0.386±0.030	NS	- 9.0

a. Activity is expressed in μMoles ATP hydrolyzed per milligram tissue protein per hour at 37°C (Mean±S.E.). Triplicate determinations were made for each tissue from 5 control and 6 experimental trout.

b. Not significant ($P<0.05$).

c. Inappropriate statistic due to the large standard error relative to the small control mean.

Tissue chromium levels of control and experimental rainbow trout exposed for two days to 2.5 mg hexavalent Cr/l are presented in Table 2. Control trout showed chromium levels ranging from 0.114 ± 0.020 μg Cr/gm wet weight in liver to 0.218 ± 0.046 μg Cr/gm wet weight in kidney. Relative mean chromium levels in different

control tissues were: kidney>intestine>gill>liver. After 48-hours exposure to hexavalent chromium, the chromium content of all tissues increased with the highest accumulation occuring in gill and kidney. Relative mean chromium levels in tissues after exposure were: kidney>gill>intestine>liver.

TABLE 2

Tissue chromium levels in unexposed rainbow trout and those exposed to 2.5 mg Cr/l for 2 days.[a]

Tissue	Controls[b]	Chromium Exposed[c]	% Increase
Kidney	0.218±0.046	2.164±0.721	891
Intestine	0.181±0.038	0.579±0.150	220
Liver	0.114±0.020	0.544±0.119	384
Gill	0.156±0.040	2.141±0.245	1275

a. Values (Mean ± S.E.) are expressed as µg Cr/gm wet weight.

b. Sample number = 8.

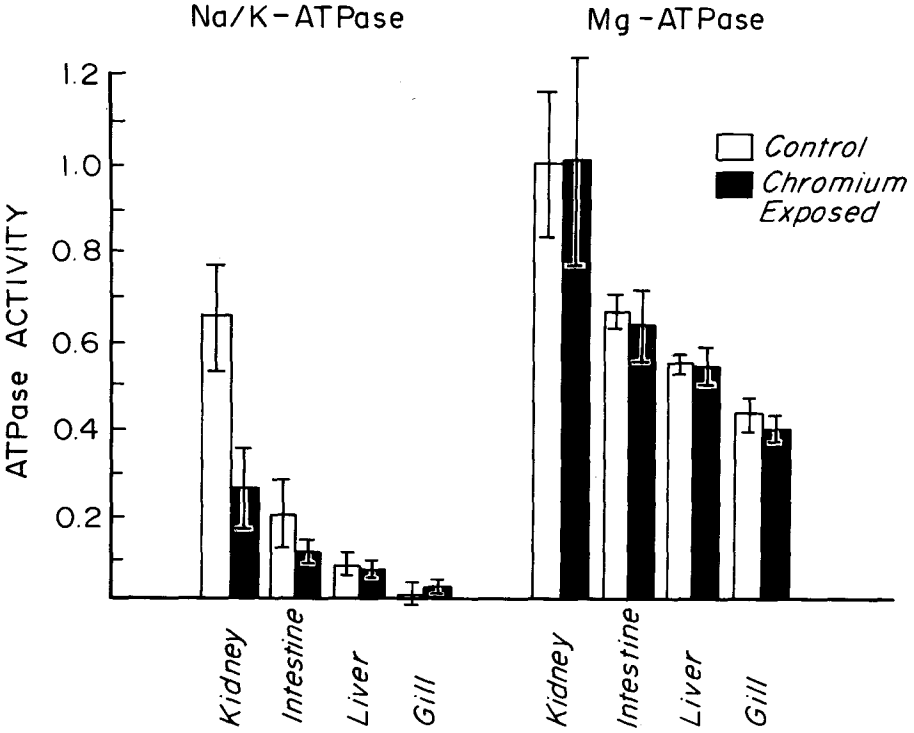
c. Sample number = 6.

Control Na/K-ATPase activity values for trout tissue are shown in Table 1 and Figure 1. The relative mean values for the tissues studied were: kidney> intestine>liver>gill. The level of Na/K-ATPase activity paralleled the level of osmoregulatory sodium transport in each tissue except for the gill; apparently sodium transport in the gill is also regulated by a sodium dependent ATPase (HOCHACHKA and SOMERO 1973).

The relative magnitude of mean Mg-ATPase activity values for the same tissues from control trout (Table 1, Figure 1) were similar to Na/K-ATPase values, ie. kidney> intestine>liver>gill. However, the absolute differences were less between extremes (e.g. kidney and gill). The exact role of Mg-ATPase is unknown.

FIGURE I

Comparison of Na/K- and Mg-ATPase activity in tissues of untreated controls and experimental trout exposed to 2.5 mg Cr/1 for 2 days.



DISCUSSION

The principle purpose of this study was to determine whether or not Na/K- and Mg-ATPase activities are altered in rainbow trout that were exposed to low levels of hexavalent chromium. Our data indicate that decreased Na/K-ATPase activity occurred in all the tissues studied except for the gill. The kidney, which had the highest control Na/K-ATPase activity, showed significant chromium inhibition. In the other tissues, the degree of chromium inhibition appeared to be directly related to its susceptibility, i.e. the initial level of Na/K-ATPase activity. At the same time, Mg-ATPase activity was unaffected by the accumulation of chromium in the tissues. These results may partially explain the detrimental effects of

hexavalent chromium on fish.

Other studies have been done in an attempt to determine the mechanism of chromium toxicity on fish. STOKES and FROMM (1965) reported that glucose transport by caecal and midgut sections from trout exposed to 2.5 mg/l chromium for one week were, respectively, 40 and 32% lower than controls. They suggested that the decrease was primarily the result of an inhibition of the entry of glucose into the epithelial cells caused by the presence of chromium in tissues. In view of the evidence that has accumulated supporting the dependence of sugar and amino acid transport on simultaneous transport of sodium (STEIN 1967) and considering the results of the present investigation, it is possible that the effect of chromium on glucose transport observed by STOKES and FROMM (1965) may have been due to the inhibition of intestinal Na/K-ATPase activity.

In another study by FROMM and STOKES (1962) on the effect of hexavalent chromium on oxygen consumption, they noted a tendency for water content to increase in tissues from fish exposed to chromium. They suggested that this phenomenon may have been due to kidney damage. Since the kidney has high Na/K-ATPase activity and the water content would vary with the regulation of sodium and potassium, perhaps the increase in the tissue water content observed by them was due to the inhibition of kidney Na/K-ATPase.

The inhibition of enzyme activity by chromium may be brought about by the binding of trivalent chromium to protein. GRAY and STERLING (1950) and GROGIN and OPPENHEIMER (1955) have suggested that hexavalent chromium may bind to protein *in vivo* after reduction to the trivalent state. This phenomenon may also partially explain how the chromium becomes trapped in certain tissues and organs. However, this does not adequately explain why in our studies inhibition occurred to Na/K-ATPase, but not to Mg ATPase activity. Further studies are needed to determine the mechanism of chromium inhibition of Na/K-ATPase activity.

In summary, the results reported in this paper indicate that inhibition of Na/K-ATPase activity may occur in several tissues of the rainbow trout following *in vivo* exposure to hexavalent chromium. Inhibition of this ion transport enzyme, which is intimately coupled to osmoregulation, may partially explain the toxicity of hexavalent chromium on fish.

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