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Metabolic Levels in Dogfish Gill Tissue After Zinc Treatments*

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Dogfish were subjected to acute (80 ppm/24 hours) and subacute (10 ppm/21 days) zinc treatments before measuring the ATP, glycogen, protein, and lactate levels. Acute treatment varies significantly the levels of all the metabolites tested while only protein content is significantly lower after subacute treatment. The results are discussed in relation to the hypoxia effect produced by the metal and the related responses of the fish.

Key words: Zinc gills, Gill metabolism.

Zinc has been known to be an essential metal acting as a catalytic or structural component and having specific functions indispensable for life. Zinc is involved in numerous enzymes related with energy metabolism as well as in transcription and translation. However, when zinc occurs at higher concentrations than normal it can act as a pollutant, producing a wide range of effects at molecular, metabolic, and physiological levels. Studies of zinc effects on fish have been published describing important changes in respiratory physiology including increase of ventilatory rhythm (10, 21), decrease of oxygen consumption (20), increase of coughing (18), and decrease of gill tissue oxygen consumption (22, 23). Because of these effects, some authors suggest that the main action of zinc would be hypoxic leading to compensatory responses of hyperventilation. Several studies (3, 9) have found increments of lactate levels after zinc intoxication, suggesting an increase in the utilization of anaerobic pathways as another compensating mechanism against hypoxia. The purpose of the present work is to find out whether metabolic level changes related to anerobic metabolism

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occur in several tissues after different zinc treatments.

Materials and Methods

Thirty-three dogfish *Scyliorhinus canicula* L. were used. Fish were collected from the coast of Barcelona (Spain). After a period of 3 weeks in an open seawater circulation tank, the fish were transferred to a closed circulation tank for treatment. 8 fish were treated acutely (80 ppm Zn for 24 hours), 17 fish subacutely (10 ppm for 21 days) and 8 fish were used as controls. Zinc was administered as zinc sulphate added to seawater. Seawater and zinc solutions were replaced every 72 h to prevent changes in water characteristics from fish excretion or metal reaction to the container (7,*8).

After killing the fish by decapitation, gill tissue was excised and prepared for biochemical analysis. Samples for glycogen were weighed and immediately digested during 20 min in 30 % KOH at 100° C. Glycogen was purified by precipitation with 95 % ethanol, centrifugation and redissolution with distilled water. Diluted glycogen was measured by the anthrone method (17). Samples for ATP and lactate were weighed and homogenized in 10 % Tris-HCl at 4° C and centrifuged at 5,000 rpm. Lactate levels were analyzed spectrophotometrically by means of NADH produced in an enzymatic reaction with lactate dehydrogenase (13). ATP levels were recorded in a Biolumat (Berthold) apparatus by means of the firefly luciferine-luciferase method (4). Protein content was measured by means of a modification (1) of the LOWRY method (14) with pholin reagent. Student-t tests were used for assessing differences between control and treated groups. 0.05 was taken as the level at which significance was accepted.

Results

Different responses were found between the two treatments (figure 1), acute treatment producing changes in all

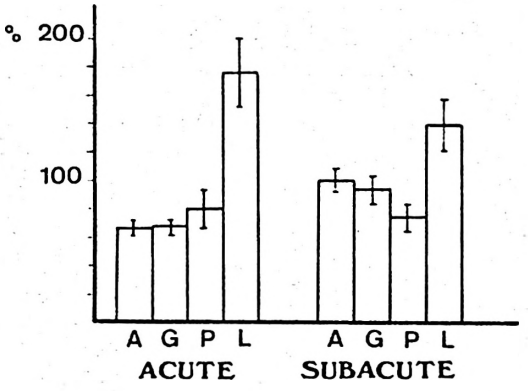

Fig. 1. *Relative levels of ATP (A), Glycogen (G), Protein (P) and Lactate (L) after treatments.* Meand and standard error of the mean.

Table I. *Metabolic levels mean and standard deviation (m + s.d.) following acute (80 ppm Zn/24 hours) and subacute (10 ppm Zn/21 days) treatments.*
* Significant difference ($p \leq 0.05$) respect to the control value.

	GLYCOGEN (mg/g w.wt)	PROTEIN	LACTATE (μmol/g w.wt)	ATP
Control	2.40 ± 0.65	17.08 ± 6.80	1.26 ± 1.02	3.26 ± 1.10
Acute	1.39 ± 0.36*	10.12 ± 3.17*	2.53 ± 0.60*	1.61 ± 0.20*
Subacute	2.19 ± 0.58	10.97 ± 5.66*	1.71 ± 0.94	3.25 ± 0.97

the four measured metabolites. Both treatments affected significantly the levels of protein in gill tissue, but only acute treatment changed significantly the levels of ATP, glycogen and lactate as can be seen in table I. Although some differences were found in mean lactate levels (30 % respect to the control mean), they were not significant. No effect of subacute treatment on ATP and glycogen levels was observed.

Discussion

Previous work on zinc toxicology of dogfish in our laboratory showed that 80 ppm was the 50 % lethal concentration at 48 hours (5). At such a concentration, metal accumulation and tissue damage can be seen, while tissue respiration is inhibited (22). At the metabolic level a significant imbalance is produced. ATP content of gill tissue decreases and glycogen, protein, and lactate levels also change as shown in the present work. Reduction in ATP content (about 25 %) has been found after 7 days of Cu treatment on *Mytilus edulis* (25) while ATP decrease caused by hypoxia has also been described in the same species (26). After an ATP decrease induced by anoxia in *Cardium edule* gill tissue a higher rate of ATP utilization, especially during the first hours of anoxia has been suggested by other authors (16). In accord with them, we have found ATP content reduction after tissue hypoxia caused by zinc treatment. Lactate concentration greatly increases after zinc acute treatment from an increase in anaerobic metabolism compensating the hypoxic effects of the metal. Most authors (2, 9, 16) have found a significant increase of lactate levels after zinc treatment of hypoxia. Increase of glucogen utilization and decrease of glycogen content in tissues after zinc exposure have also been found (9), suggesting that glycogen is used at higher rates in the glycolytic pathway when hypoxic conditions are present and an extra energetic supply is required. After 21 days of 10 ppm Zn treatment only the protein levels were significantly lower than controls. Similar results have been found by other authors suggesting that such action is exerted by heavy metals interfering with uptake of aminoacids and protein synthesis (25).

Even if the precise localization of zinc effects remains still unclear, some authors have described effects at the molecular level that might partially explain the action of the metal. Electron transport inhibition in mitochondria (12) and other biochemical studies (15) suggest that Zn binds to NADPH inhibiting the drug oxidizing system. A high inhibition of respiration in fish liver mitochondria has also been described (27) while several authors (19) have discussed the alternative hypothesis of the metals acting by attaching themselves to protein sites either normally free or occupied by other metals, resulting in impairment of their physiological activity (6, 24). However, most of these effects have been found after *in vitro* treatment. There is no consistent relationship between *in vivo* and *in vitro* effects of metals on enzymes (11) since high amounts of metals are necessary to produce those effects *in vivo* (27). While some authors (19) mostly emphasize the gill damage produced by zinc, suggesting an impairment of gill structure, other authors emphasize the hypoxia effect after zinc treatment (3). Nevertheless, both interpretations might yet be compatible, since damage to the tissues would impair the oxygen diffusion process to them, thus creating a hypoxic situation, an energetic imbalance and consequent responses of increased anaerobic metabolism as our results with dogfish show.

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Resumen

Se determinan los niveles de ATP, glucógeno, proteína y lactato en peces lija después de tratamientos agudo (80 ppm/24 horas) y subagudo (10 ppm/21 días) con zinc. El tratamiento agudo modifica significativamente los niveles de todos los metabolitos, mientras que sólo los niveles de proteína descienden significativamente en el tratamiento subagudo. Los resultados se discuten en relación al efecto hipóxico ejercido por el metal y las respuestas consiguientes que se producen en el pez.

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