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Mechanisms for Zinc Acclimation in Freshwater Rainbow Trout

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ABSTRACT

A sublethal effect of zinc exposure in freshwater fish is the inhibition of calcium uptake. However, calcium also inhibits zinc uptake and this inhibition has been found to be competitive (Spry, D.J. & Wood, C.M. (1989). J. Exp. Biol., **142**, *425-46).*

The subject of the acclimation of aquatic animals to metals is well researched, but there is, as yet, no simple answer as to how this acclimation is accomplished. The objectives of the present study were to characterize the interactions of zinc on calcium uptake kinetics, and to investigate if a restoration of calcium influx was associated with zinc acclimation. In addition, the possible involvement of metallothionein and a reduced zinc influx in the acclimation process was studied.

INTRODUCTION

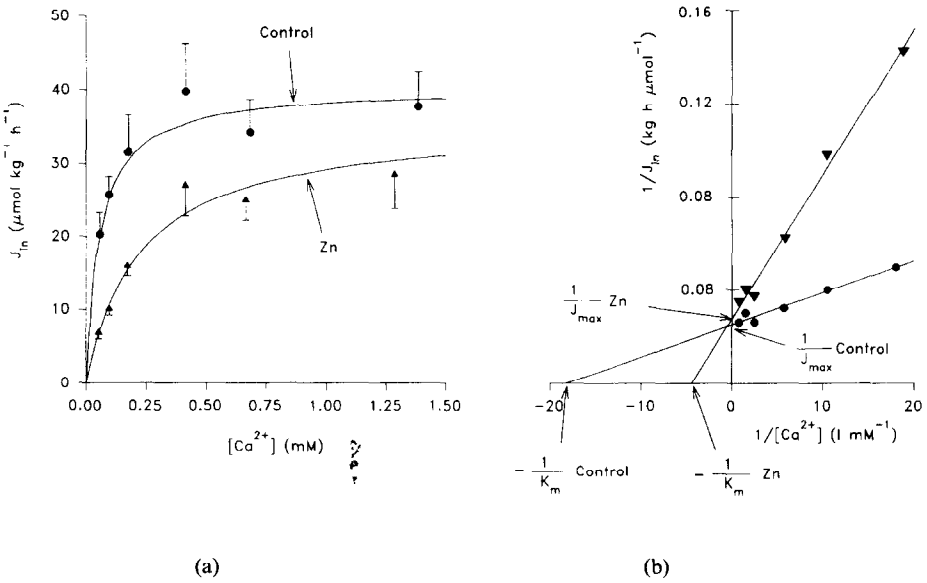
One of the most important sublethal effects of zinc exposure in freshwater fish is an inhibition of calcium uptake. It has been shown by Spry & Wood (1985) that the net calcium uptake was completely abolished during exposure to sublethal zinc. However, calcium has also been found to inhibit zinc uptake, the inhibition being competitive (Spry & Wood, 1989). The nature of the interference by zinc on calcium uptake is unknown.

There is an extensive literature on the acclimation of aquatic animals to metals, but no simple answer as to how the acclimation is accomplished (McDonald & Wood, 1993). Aluminium and copper both have effects on the branchial influx of sodium (Laurén & McDonald, 1987; McDonald *et al.*, 1991). It has been shown that acclimation to these metals occurs concomitantly with a restoration of branchial sodium influx, indicating that this might be an adaptive mechanism responsible for the acclimation. This study aims to characterize the interactions of zinc on calcium uptake kinetics, and to investigate if a restoration of calcium influx is associated with zinc acclimation. The possible involvements of metallothionein and a reduced zinc influx in the acclimation process are also studied.

Two separate experiments were performed in which juvenile rainbow trout were exposed to 150 ppb zinc ([Zn] = 2.3 μM) in hard water ([Ca] = 1.0 mM). An unexposed group held under identical conditions served as a control. In the first experiment (Hogstrand *et al.*, 1994*a*), unidirectional calcium uptake kinetics was measured (using ^{45}Ca) throughout an exposure period of two months. The LT_{50} (at lethal [Zn] = 3.4 ppm) was determined in the middle and at the end of the acclimation period, and unidirectional zinc influx (using ^{65}Zn) together with levels of copper, zinc and MT (by specific RIA) in gills and liver were analyzed after two months of exposure. The exposure conditions in the second experiment were the same as in the first, but the experimental period was one month (Hogstrand *et al.*, 1994*b*). In the second experiment both the true and apparent values for the number of calcium uptake sites (J_{max}) and their relative affinities (K_m) for calcium were determined throughout the experiment (i.e. J_{max} and K_m both in the absence and the presence of the inhibitor, zinc). Zinc influx, plasma calcium, LT_{50} ([Zn] = 3.4 ppm), and gill zinc content were measured during the exposure period.

Exposure to zinc initially caused hypocalcemia but the plasma calcium level was restored within a week. In the first experiment an increased resistance to Zn in the LT_{50} test was observed after 27 days of exposure, and then throughout the rest of the experiment. Interestingly, no increased zinc resistence was developed in the second experiment. However, the plasma concentration of calcium was normalized within a week. Apparently, physiological adaption to zinc, in terms of restored plasma calcium levels, and an increased tolerance to zinc in acute toxicity tests, are achieved by separate mechanisms. The K_m for calcium influx of unexposed fish varied between 25 and 100 μM, in all cases well below the acclimation calcium concentration of 1.0 mM. The presence of zinc in the water increased the K_m for calcium influx with little effect on J_{max} (Fig. 1), indicative of simple competitive inhibition. This further substantiates previous evidence that zinc and calcium directly compete for the same uptake sites (Spry & Wood, 1989). Initially, there was also a small reduction in the J_{max} (slight non-competitive component) which was restored after about a week. After one day of zinc exposure, the effect on the apparent K_m was abolished and the true K_m was dramatically decreased (Fig. 2). This could be interpreted as a response to the lesser amount of calcium available for the fish. Thus, the affinity of the uptake sites was increased in an attempt to compensate for the reduced calcium uptake. However, after about a week of exposure, the K_m was increased again and both the apparent and the true K_m stabilized at levels well above the control (Fig. 2). Thus, the fish were adapting to zinc without a concomitant restoration of the K_m for calcium influx. It was found that J_{max} rather than K_m was the pivotal factor determining the calcium influx at the water [Ca^{2+}] in which the experiments were performed. Since the J_{max} was restored within a week, most of the calcium influx was restored at this point, explaining the recovery of plasma calcium.

After one day of zinc exposure, the zinc uptake at 150 ppb was not different between the groups. At day 4 and 7, there was a more rapid influx of zinc in exposed fish, compared to the controls. Later in the experiment, however, the zinc influx decreased in exposed fish to eventually reach values that were about half that of the control. The competitive interactions between the influxes of zinc and calcium, and a covariance between zinc uptake and the kinetic uptake parameters for calcium, suggest that there may be a link between the decreasing affinity of the uptake sites for calcium and the reduced uptake of zinc. In the first experiment,



(a) (b)

Fig. 1. (a) Unidirectional calcium uptake kinetics in hardwater acclimated ([Ca^{2+}] = 1.0 mM) Rainbow trout and the effect of sublethal waterborne zinc (150 ppb = 2.3 μM) on the calcium uptake. Filled circles show the influx of calcium in control fish, on day 50 of experiment 1, at different concentrations of external calcium. Triangles show the calcium uptake kinetics in fish exposed for 50 days to sublethal zinc. Each point represents the mean of eight fish, and vertical bars one-way SEM. (b) Lineweaver–Burk plot of the data shown in Fig. 1(a). Note the large increase in K_m and small reduction in J_{max} in response to zinc exposure.

there were no changes in hepatic or branchial levels of zinc, copper or MT in zinc exposed fish. A minor increase in the branchial zinc level was found in the second experiment after exposure to zinc for one month, but not before. The content of MT in the gills was low and this heavy metal-binding protein only bound a minute fraction of the Zn present in the gills.

In conclusion, acclimation to zinc in freshwater-adapted rainbow trout can develop without any detectable increase of zinc accumulation in the gills or liver. Consequently, acclimation to zinc does not necessarily involve induction of MT. The inhibition of the calcium influx by zinc is mainly competitive in its nature, and persists during chronic exposure, indicating that zinc and calcium compete for the same uptake sites. There is, however, a minor non-competitive component involved, which can be restored within a week. The restoration of J_{max} is paralleled by a normalization of plasma calcium. During long-term exposure to zinc, the affinity of the calcium uptake sites is chronically depressed but this has little effect on the J_{max} for calcium at the water hardness used in the experiment. An important difference between zinc-adapted fish and the controls is that the former have a decreased rate of zinc influx, compared to the controls. Thus, it is speculated by these authors that the fish are able to regulate the uptake of zinc separately from that of calcium by selective manipulation of K_m for a mutual Ca^{2+}/Zn^{2+} carrier, so as to markedly reduce zinc influx without greatly altering the

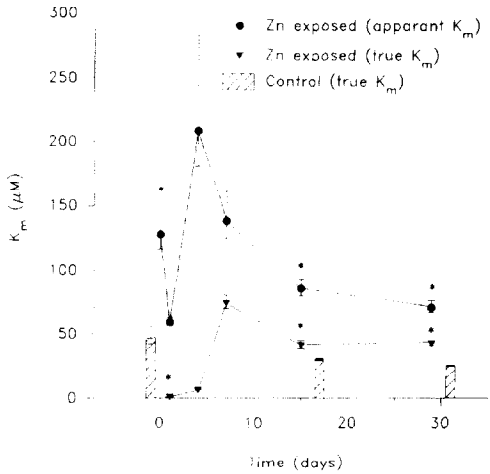


Fig. 2. Alterations in the affinity constant (K_m) for unidirectional calcium influx in hardwater acclimated ($[Ca^{2+}] = 1.0$ mM) Rainbow trout during exposure to zinc for one month. Bars, circles and triangles show the mean values, derived from the regression lines of Lineweaver–Burk plots, and the vertical bars indicate the assymetrical SEM obtained from the SD of the regression lines ($n = 6$ groups of 8 fish each). The bars show the K_m for calcium influx of control fish, whereas circles represent the apparent K_m (in the presence of zinc) and triangles represent the true K_m (in the absence of zinc) of calcium influx in zinc-exposed fish. An * denotes a statistically different ($p < 0.05$, Students t-test) value compared to the closest (in time) control. Data from exposed fish, obtained on day 4 and day 7, were not analyzed statistically.

influx of calcium. This may be the mechanism responsible for the adaptation to increased levels of waterborne zinc.

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REFERENCES

Hogstrand, C., Wilson, R.W., Polgar, D. & Wood, C.M. (1994*a*). *J. exp. Biol.* (in press).
Hogstrand, C., Reid, S.D. & Wood, C.M. (1994*b*). *J. exp. Biol.* (submitted).
Laurén, D.J. & McDonald, D.G. (1987). *Can. J. Fish. Aquat. Sci.*, **44**, 105–11.
McDonald, D.G. & Wood, C.M. (1993). In *Fish Ecophysiology*, eds J.C. Rankin & F.B. Jensen. Chapman and Hall, London, pp. 297-321.

McDonald, D.G., Wood, C.M., Rhem, R.G., Mueller, M.E., Mount, D.R. & Bergman, H.L. (1991). *Can. J. Fish. Aquat. Sci.*, **48,** 2006–15.
Spry, D.J. & Wood, C.M. (1985). *Can. J. Fish. Aquat. Sci.*, **42,** 1332–41.
Spry, D.J. & Wood, C.M. (1989). *J. Exp. Biol.*, **142,** 425–46.