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Effects of Al and Cd on Vitellogenin mRNA Induction by Estradiol-17β in the Primary Culture of Hepatocytes in the Rainbow Trout, *Oncorhynchus mykiss*

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Introduction

Recently, industrial activities have increased atmospheric concentration of sulfur and nitrogen oxides, resulting in acidification in the environments. In addition, acidification accelerates the mobilization of metals that are toxic to fish and increases their concentrations in the aquatic environment. Increased metals may interfere with reproductive physiology in fish. Al and Cd are such metals that impaired the production of Vitellogenin (VTG), a egg yolk precursor proteins. Impaired oogenesis due to poor accumulation of egg yolks (Lee and Gerking, 1980), a reduction in egg number (Runn *et al.*, 1977), and incomplete hatching (Runn *et al.*, 1977) have been reported in acidic waters, resulting in recruitment failure. However, it remains unclear how these metals interfere with the process of VTG synthesis.

The present study was undertaken to determine at which levels these metals interfere with VTG induction by Estradiol-17β in the primary culture of hepatocytes in the rainbow trout.

Materials and Methods

Hepatocytes were precultured for 2 days and then estradiol-17β (E_2, 2×10^{-6} M in 3$\mu\ell$ of 95% ethanol) and Al ($AlCl_3$ in 3$\mu\ell$ of redistilled water) or Cd ($CdCl_2$ in 3$\mu\ell$ of redistilled water) were simultaneously added to the incubation medium.

Final metal concentrations were 10^{-6}, 10^{-5}, 5×10^{-5}, and 10^{-4} M for Al and 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M for Cd. The effects of these metals on VTG production were examined 5 days after addition, during which time the media were changed daily. Control cultures received the equivalent amount of the solvents only. Media and hepatocytes were then analyzed by SDS-PAGE and Northern blotting for VTG and VTG mRNA, respectively. Effects of Al and Cd removal on the metal-induced inhibition of VTG production were also examined. Hepatocytes were cultured with E_2 and Al (10^{-4} M) or Cd (10^{-6} M) for 3 days and then these metals were removed from the media (defined as Day 0). The cultures were continued for another 7 days.

Results

These metals had no appreciable effect on the viability of hepatocytes in culture. However, Al and Cd interfered with VTG production and VTG mRNA expression. Al reduced VTG production in a concentration-dependent way and a significant reduction occurred at Al concentrations greater than 5×10^{-5} M. VTG mRNA expression also decreased with a negative correlation with Al concentrations (r = -0.98). The inhibition of VTG production by Cd was not concentration-dependent. This metals markedly inhibited VTG production and VTG mRNA expression at 10^{-6} M. The Al-induced inhibition of VTG production was restored 7 days after Al removal, but the Cd-induced inhibition was not restored. These results suggest that Al and Cd inhibit VTG production at the transcriptional level to reduce VTG mRNA expression by different mechanisms.

References

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