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Metallothionein Gene Expression in Arctic Char (*Salvelinus alpinus*) Following Metal and PCB Exposure

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ABSTRACT

*Full length sequences of two Arctic char (*Salvelinus alpinus*) metallothionein (MT) cDNAs, corresponding to MT-A and MT-B, were isolated from a λ-ZAP hepatic cDNA library. The two MT isoforms showed high similarity to rainbow trout and Atlantic salmon MT, with the coding sequences being 100% homologous to rainbow trout MT. Following characterization the MT cDNA was used to study the inducibility of Arctic char MT mRNA. Basal level expression was determined for 11 different tissues and it was observed that liver and immature gonads expressed the highest levels of MT mRNA. The inducibility of MT mRNA, by cadmium, 17β-estradiol (E2) and three PCBs (2',4',6'-trichloro-3-biphenylol, 2,2',4,6,6'-pentaCB and 2,3,3',4,4',5',6-heptaCB) was thereafter determined for liver, kidney and brain. While cadmium induced MT gene expression in all three organs, E2 treatment did not affect the MT mRNA levels. All three PCBs were found to result in reduced basal level MT mRNA expression.*

INTRODUCTION

Metallothioneins (MTs) are low-molecular weight proteins with a high percentage of cysteine residues. These residues are involved in the binding of both monovalent and divalent cations (Olsson, 1993). The proposed function of MT is both in homeostatic control of essential transition metals, such as Zn and Cu, and in the detoxification of toxic heavy metals, such as Cd and Hg (Zafarullah *et al.*, 1989; Olsson, 1993). Administration of cadmium to fish increases hepatic MT levels through induction of MT gene transcription and this is thereafter followed by binding of Cd to newly synthesized MT (Chan *et al.*, 1987; Olsson *et al.*, 1989).

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Two MT isoforms, encoded by the MT-A and the MT-B gene have previously been isolated from rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) (Bonham *et al.*, 1987; Kille and Olsson, 1996*a,b*). In studies on rainbow trout it has been observed that the MT-A gene is directly regulated by metals and free radicals while the MT-B gene is mainly metal responsive (Zafarullah *et al.*, 1988; Olsson *et al.* 1995*a*). Indirect regulation of these genes may, however, be accomplished by a number of other substances (Olsson, 1993).

Indirect inhibition of MT transcription and translation has been observed following estradiol-17β, (E2) treatment of rainbow trout (Olsson *et al.*, 1995*b*). Certain environmental contaminants, including para-hydroxylated PCBs, have been shown to mimic estrogenic responses in a range of organisms (Toppari *et al.*, 1996). It is, however, not known if these estrogenic effects include down-regulation of MT.

The aim of this paper was to isolate and characterize Arctic char (*Salvelinus alpinus*) MT cDNA and to determine the basal level expression and regulation of MT mRNA in different organs.

MATERIALS AND METHODS

RNA was isolated from untreated arctic char liver and used to construct a λ-ZAP cDNA library. A digoxigenin labeled zebrafish MT cRNA probe (Kille and Olsson, 1996*c*) was used to screen the library. From a total of 100 000 plaques, several positive clones were picked and sequenced and full length copies for both MT-A and the MT-B mRNA were thereby obtained.

Juvenile Arctic char were held in aquarium at 10°C and a 14 h light and 10 h dark period. The fish were not fed during the experiment. For basal level detection of MT mRNA, 11 organs were collected from four fish. At the start of the induction experiment 32 fish were separated in groups of four and injected with peanut oil (control fish), Cd (0.5 mg kg^{-1}), E2 (10 mg kg^{-1}), 2′,4′,6′-trichloro-3-biphenyl (OH–PCB; 5×10^{-6} moles kg^{-1}), 2,2′,4,6,6′-pentaCB (PCB No.104; 5×10^{-6} moles kg^{-1}) or 2,3,3′,4,4′,5′,6-heptaCB (PCB No.190; 5×10^{-6} moles kg^{-1}). Liver, brain and kidney were sampled after 1, 3 and 7 days for Cd and after 7 days for controls, E2 and PCBs. At sampling, the tissues were frozen in liquid nitrogen and thereafter immediately stored at −80°C.

Total RNA was extracted following the method of Chomczynski and Sacci (1987), and the intactness of the RNA was checked on 1.2% agarose gel electrophoresis. Single stranded RNA probe for Arctic char MT-A was labeled with digoxigenin and used to detect total levels of MT-A + MT-B transcript. Hybridization and detection of MT mRNA was performed as described earlier (Olsson *et al.*, 1995*b*). The levels of MT mRNA in control liver was arbitrarily set to 1.

RESULTS AND DISCUSSION

The coding sequences of Arctic char MT-A and MT-B (Olsson *et al.*, 1997*a,b*) were found to share high homology with both rainbow trout and Atlantic salmon. In the coding region, Arctic char MT-A share 100% homology with rainbow trout MT-A while Arctic

char MT-B deviates in three bases from rainbow trout MT-B. The deduced protein sequences show no amino acid changes when compared to the rainbow trout sequences. In the 5′ and 3′ untranslated regions there is a higher degree of variation between these genes.

Determination of the relative basal levels of MT mRNA in different organs of Arctic char, showed that the liver and immature gonads expressed the highest MT mRNA levels, while the muscle levels were non-detectable (Fig. 1). Gonads of immature rainbow trout have earlier been shown to express high levels of MT mRNA (Zafarullah *et al.*, 1989; Olsson *et al.*, 1990).

The results of different treatments on brain, liver and kidney MT mRNA levels are presented in Fig. 2. Both basal and induced MT mRNA levels were highest in liver and lowest in brain. Of the tested substances only Cd induced MT. There were temporal differences in Cd induced MT mRNA expression in the different tissues. Thus, maximal MT mRNA levels were obtained already on day 1 in liver, on day 3 in kidney and on day 7 in brain. These differences may reflect the movement of Cd through different compartments/organs of the fish. It is, however, interesting to note that Cd was capable of inducing brain MT transcription. While E2 had no effect on MT mRNA levels, treatment with the different PCBs resulted in lowered basal levels of MT mRNA in all organs. The lack of induction of MT mRNA by E2 is in accordance with earlier studies on rainbow trout (Olsson *et al.*, 1989, 1995*b*). The mechanism behind the PCB mediated reduction of MT mRNA remains to be determined.

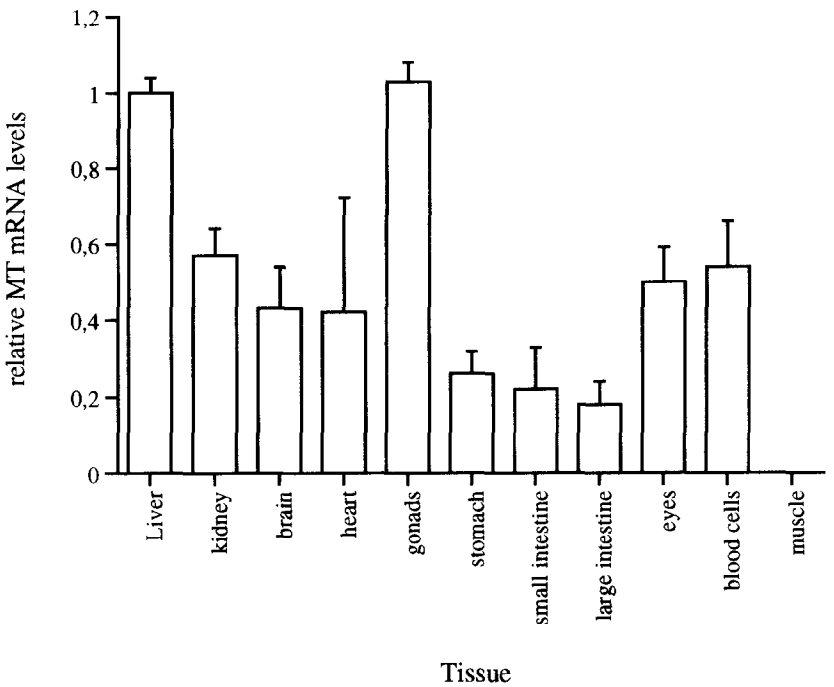

Fig. 1. Basal level expression of MT mRNA in different tissues of Arctic char. The hepatic MT mRNA level was arbitrarily set to 1. The results are presented as mean ±SD ($n=4$).

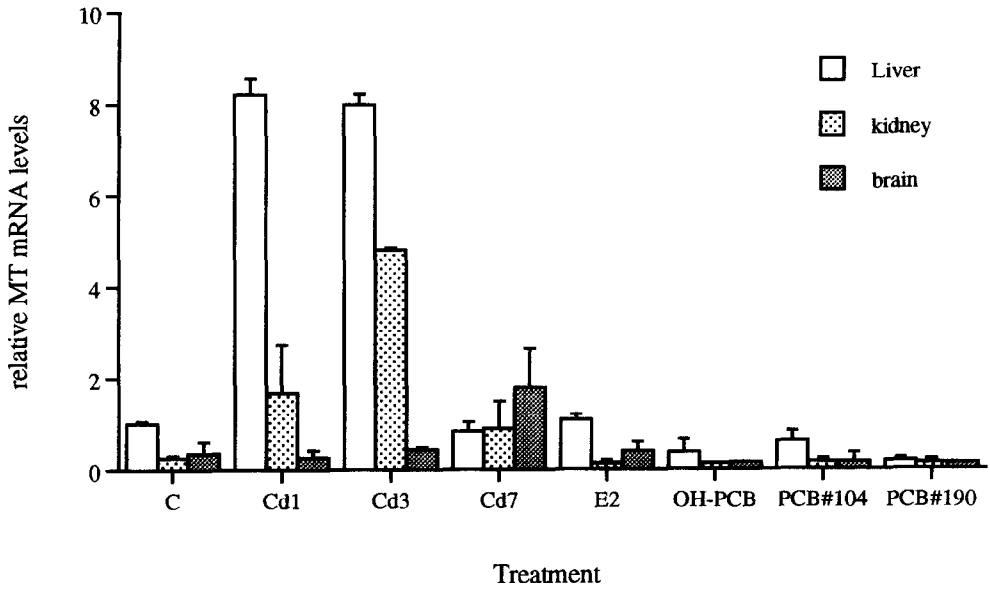

Fig. 2. Effect of Cd (sampled 1, 3 and 7 days after injection) and E2, OH–PCB, PCB No.104 and PCB No.190 (sampled 7 days after injection) on the MT mRNA levels in liver, kidney and brain of Arctic char. The liver control levels were arbitrarily set to 1. The results are presented as mean ± SD ($n = 4$).

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