PARTIAL CHARACTERIZATION OF HEPATIC BINDING PROTEINS IN THE LITTLE SKATE, RAJA ERINACEA

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Metallothioneins (MT) are low molecular weight, cysteine rich proteins highly concentrated in the liver, kidney,and intestine which play significant roles in metal detoxification (see Dunn, et al, Proc. Soc. Exp. Biol. Med. 185:107-119, 1987). Experiments using cell lines selected for high versus low and reduced MT gene expression suggest a link between the loss of ability to detoxify metals with low MT gene expression. Cell lines which overproduce MT become highly resistant to cadmium (Karin et al, P.B.A.S. (USA) 80:4040-4044, 1983). Limited data suggest that MT's detoxify metals in intact animals (Johnson et al, Hepatology 1:243-248, 1981; Mehra and Bremmer, Biochem. J. 219:539-545, 1984) including teleost fish where elevated levels of MT occur in proportion to copper levels (Roch and Carter, Comp. Physiol. Biochem. 77C:71-75, 1984). Thus MT synthesis may be a reflection of adaption to and detoxification of heavy metals, and may be a good indicator of toxicity levels in marine environments.

Although adrenal steroids and other hormones paticipate in the regulation of MT synthesis in mammals, nothing is known of the role of sex hormones in seasonally breeding marine vertebrates on MT synthesis. In addition the potential of sex hormones to influence MT levels and hence sensitivity to toxic metals is unknown. Here we describe preliminary studies of the characterization of cadmium binding proteins in the liver of the marine skate, Raja erinacea. Animals were sacrificed by cervical section, the liver removed to ice cold elasmobranch saline and minced. Minced tissues were washed three times with saline, homogenized with a Brinkman PT-10 Polytron and cytosol obtained by centrifugation at 100,000 X G in a Beckman ultracentrifuge. The cytosolic extracts were subjected to gel filtration and ion exchange column chromatography and SDS-PAGE electrophoresis. Prior to analysis, the extracts were labelled with Cd-109 as a tracer for the proteins. Gel filtration on Sephadex G-50 revealed two protein bound cadmium peaks, one eluting near the void volume and the other eluting with an approximate molecular weight of 8 KD. This 8 KD protein adsorbs to DEAE-Sepharose and elutes at0.18M KCl. This protein is similar in size and displays similar ion exchange chromatography properties as other vertebrate MT's. DEAE-Sepharose chromatography of the void volume reveal two proteins, one of which binds to the column and another which is collected in the wash-through. Neither of these proteins adsorb to CM-Sephadex. SDS-PAGE Cd-109 blots reveal two proteins of Mr of 42 and 50 KD, both of which appear near the void volume of the G-50 column. The failure to detect the 8 KD protein on the blots may reflect the difficulty of blotting cysteine rich proteins from SDS-PAGE gels under basic conditions, and further work is being done to correct this problem. The 8 KD protein described here was partially purified by DEAE-Sepharose and gel filtation chromatography and appears as a single band on Coomassie blue stained gels, suggesting one species.

These studies suggest that the skate liver synthesizes MT's and other metal binding proteins. In on going work we are assesing MT production in response to cadmium challenge in this species using HPLC. Sex differences and hormonal effects on MT production are also under investigation.

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