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Effects of 3-Methylcholanthrene on the Hepatic Microsomal Enzymes in a Teleost, *Tilapia aurea*

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Polynuclear aromatic hydrocarbons (PAH) cause induction of the mixed function oxidases (MFO) and the electron transport components (ETC) in fish. The influence of xenobiotics on the MFO and ETC are numerous and have been reviewed (BEND & JAMES 1978). Studies on the formation of the benzo(a)pyrene 7,8-dihydrodiol (a precursor to the ultimate carcinogen, benzo(a)pyrene 7,8-dihydrodiol-9,10-epoxide), and covalent binding of benzo(a)pyrene metabolites to DNA in the fish have been reported. Such work has included the little skate (BEND et al. 1979), trout (AHOKAS 1979), flounder and salmon (VARANASI & GMUR 1980), sea catfish and killifish (MELIUS et al. 1980), and mullet (TAN et al. 1981a). The aryl hydrocarbon hydroxylase (AHH) activity of the MFO has been suggested as a monitor for petroleum pollution in the marine environment (PAYNE 1976). Elevated AHH activites were found in livers of tumored salamanders (BUSBEE et al. 1978) and tumored bullheads (TAN et al. 1981b), both of which were associated with sewage water. However, BEND & JAMES (1978) and KAWALECK & LU (1975) found that variations in the MFO and ETC were often species-specific, and therefore precluded any generalized predictions concerning enzymatic oxidation and reduction systems in teleosts. This paper presents the effects of 3-methylcholanthrene (MC) on the NADPH- and NADH-linked microsomal reactions, a demethylation reaction, and two hepatic enzymes not related to the electron-transport system in Tilapia aurea. The distribution of the hepatic enzymes in the soluble and microsomal fractions was compared.

MATERIALS AND METHODS

Materials. Biochemicals were obtained from Sigma Chemical Company. Aminopyrine (4-dimethylantipyrine) was purchased from Aldrich Chemical Company. The MC was supplied by Dr. J. Keith, IIT Research Institute, Chicago, Illinois. All remaining reagents were obtained from Fisher Scientific Company.

Fish Treatment. The fish were transported from Auburn University Fisheries ponds to 100 L aquaria filled with dechlorinated water and the fish were acclimated for 7 days before treatment. The aquaria were aerated and the water temperature maintained at 18 ± 1°C. One-year-old fish weighing between 150-200 g were fasted during the treatment period. A single intraperitoneal injection was administered to each fish. In the MC treatment, four fish in each experiment were injected with a 20 mgMC/kg in purified olive

oil. Control fish received olive oil only.

Microsomal Preparations. The fish were stunned with a blow on the head and the livers were removed, and rinsed in 15 mL of chilled 0.15M KCl solution. Pooled livers from each group were placed in a beaker containing 1 mL of 0.15M KCl/g liver mass, minced with scissors, and homogenized in ice in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 10,000 g at 2-4°C for 30 minutes. The 10,000 g supernatant collected was recentrifuged at 100,000 g at 2-4°C for 90 minutes. Both the microsomal supernatant (soluble fraction) and the pelleted microsome (resuspended in 0.15M KCl solution) were saved for enzyme assays.

Enzyme Assays. Protein concentrations were determined by the method of LOWRY et al. (1951) using bovine serum albumin (BSA) as the standard. NADPH-cytochrome C reductase (NADPH-CytcR) was assayed at 25°C and pH 7.4 (YONETANI 1965). Absorbance change with time was monitored at 550 mm to follow the cytochrome c reduction (ε = 19.6mM^{-1}cm^{-1}). NADH-cytrochrome b_5 reductase (NADH-Cytb$_5$R) activity was followed spectrophotometrically at 25°C and pH 7.4. (SCHELLENBERG & HELLERMAN 1958). Absorbance change with time was monitored at 420 nm for the reduction of potassium ferricyanide (ε = 1.02mM^{-1}cm^{-1}). The NASH (1953) procedure using Tris buffer (0.1M, pH 7.5) was used (KLAUNIG et al. 1979). Incubation was performed at 25°C and for 30 minutes. The hepatic glutamic pyruvic transaminase (HGPT) and hepatic glutamic oxaloacetic transaminase (HGOT) were assayed using the Sigma Kitset No. 505 measuring the activities at 505 nm, 37°C, and pH 7.5. In all the enzyme assays, the Gilford 250 spectrophotometer was used.

RESULTS AND DISCUSSION

The effect of MC treatment in Talapia hepatic microsomal enzymes is shown in Table 1. Nominal increase in enzyme activities of treated over control fish was observed in NADPH-CytcR (68%), NADH Cytb$_5$R (64%), HGPT (68%) but was much higher in HGOT (120%). Increase in APND activity was not significant.

TABLE 1. Hepatic microsomal enzymes of Tilapia from 3-methylcholanthrene treatment.

Fish[a]	NADPH-CytcR (nmole/min/mg)[b]	NADH-Cytb$_5$R (µmole/min/mg)[b]	APND (nmole/min/mg)[b]	HGPT (Sigma Frankel units/mL)[c]	HGOT (Sigma Frankel units/mL)[c]
Control	2.5±0.3	0.28±0.05	1.14±0.03	115 ± 3	115 ± 5
Treated	4.2±0.6	0.46±0.04	1.55±0.61	193 ± 12	253 ± 71

a) Pooled livers from 4 fish were used in each experiment.
b) Two separate experiments of 2-4 replicates each; mean ± SE.
c) Two separate experiments of 8 replicates each; mean ± SE.

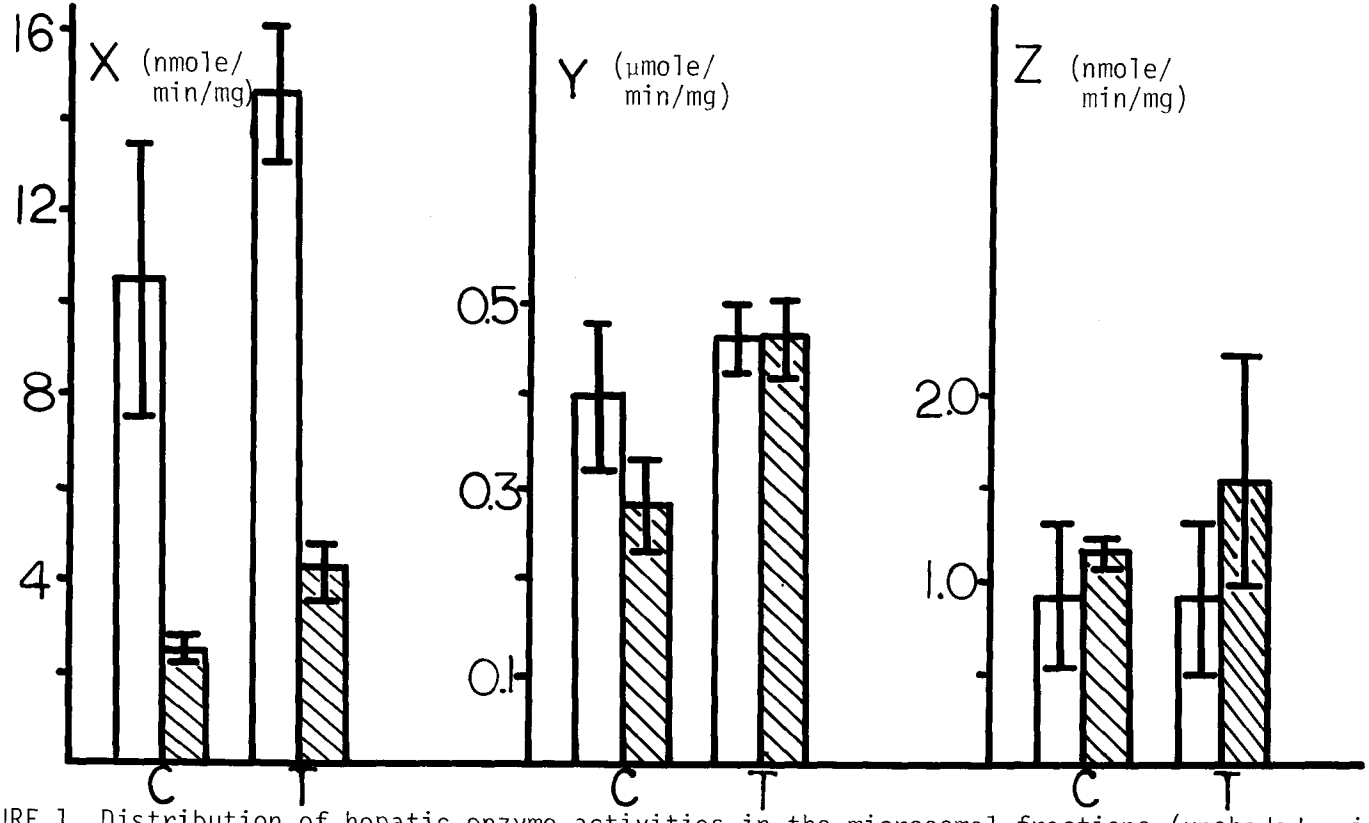

FIGURE 1. Distribution of hepatic enzyme activities in the microsomal fractions (unshaded, microsomal supernatant; shaded, microsome). The legends are: C, control fish; T, treated fish; X, NADPH-CytcR; Y, NADH-Cytb$_5$R; and Z, APND. Each bar is the mean $\pm$ SE (2 separate experiments containing 2 to 4 determinations each).

JAMES & BEND (1980) noted that NADPH-CytcR activities were unaffected by MC treatment in flounder, stingray, little skate, sheephead and dogfish shark. Similar negative findings were also reported in mullet, sea catfish and gulf killifish (MELIUS et al. 1980). The NADPH-CytcR in Talapia was unaffected by trans-stilbene oxide (TSO) at 200 mg TSO/kg treatment level (TAN & MELIUS 1981). It appeared that the MC at the 20 mg MC/kg treatment level would affect the Tilapia electron transport-related hepatic enzymes (NADPH-CytcR and NADH-$Cytb_5R$). Elevated HGOT and HGPT activities in rainbow trout near a sewage plant outflow (WIESER & HINTERLEITNER 1980) was attributed to toxic effects in the outflow. At the increased levels of HGOT and HGPT indicated in Tilapia, some hepatic damage may be expected at the 20 mgMC/kg treatment.

Figure 1 indicates the distribution of the NADPH-CytcR, NADH-$Cytb_5R$, and APND in the pelleted and soluble microsomal fractions. When ultracentrifugation was used to sediment microsomal protein, the soluble fraction has 3.5-fold more NADPH-CytcR activities than the microsome. This was observed in both fractions, these activities were equally distributed in the soluble and microsomal fractions (see Figure 1Y and 1Z).

NADPH-dependent activity was localized in the microsomal supernatant fraction of lobster hepatopancreas (BEND & JAMES 1978) and the activity is apparently not cytochrome P_{450}-dependent (JUCHAU & SYMMS 1972; ELMAMLOUK & GESSNER 1976). It is possible that the soluble fraction contained sufficient levels of glucose-6-phosphate dehydrogenase (G6PD), which would enhance the NADPH-CytcR activity. The G6PD-enhancement of the microsomal drug oxidase activity in the soluble fraction has been reported in the mammalian system (AXELROD 1956) and tested in fish (BUHLER & RASMUSSON 1968).

MC (20 mg/kg) induced the electron-transport reduction related enzymes in Tilapia with some associated hepatic damage. NADPH-CytcR activities were found to be 3.5-fold higher in the soluble supernatant than the pelleted microsomal fractions.

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