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Ultrastructural and Biochemical Effects of 3-Methylcholanthrene in Rainbow Trout

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Effluent discharged from industrial operations often contains a variety of chemicals which can be cytotoxic, mutagenic and carcinogenic in nature. Among these xenobiotic chemicals, the polyaromatic hydrocarbons (PAH) are frequently present in contaminated aquatic environments (Varanasi et al. 1989). Fish absorb these PAH and metabolize them, primarily in liver, by cytochrome P-450 (P-450) - linked mixed function oxygenases (MFO) to polar and reactive products. Increased incidences of hepatic lesions (neoplasms) were observed in fish collected from sites contaminated with high levels of PAH (Baumann 1989).

Laboratory and field studies have shown that PAH and petroleum hydrocarbons are potent inducers of MFO in various fish species (Payne et al. 1987; Smith et al. 1991). The role of MFO in the biotransformation of PAH and other lipophilic xenobiotics has made the activity of these enzymes a useful biomarker of these pollutants in aquatic species. Because MFO activities in fish tissues can also be altered by various abiotic (e.g. temperature) and biotic (e.g. age, sex) factors (Payne et al. 1987), the assessment of accompanying subcellular morphological changes could be useful in delineating induced biochemical effects. In this paper, we have studied these subcellular effects in the liver and kidney of rainbow trout experimentally exposed to 3-methylcholanthrene (3-MC), an MFO inducing agent. Ultrastructural (liver and kidney) and biochemical (liver) indices were used to demonstrate induced alterations. Such a combined structure-function assessment is rarely used to characterize MFO induction and other systemic effects in aquatic species.

MATERIALS AND METHODS

All biochemicals and benzo(a)pyrene were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of highest commercially available grade.

Mature rainbow trout (*Oncorhynchus mykiss*) weighing >270 g were used. The fish were maintained at 15°C according to standard aquatic husbandry procedures.

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All experimental treatments were carried out according to Canadian Council on Animal Care guidelines (CCAC 1984). Fish were injected intraperitoneally with a single dose of 3-MC (12.5 mg/kg body weight) in corn oil. Control fish were injected with a similar volume of corn oil. At 48 hr post-treatment, the fish were euthanized by blunt cranial trauma producing immediate unconsciousness and the liver and kidney were excised.

Thin slices (0.5-1.0 mm) of liver and posterior kidney were taken and fixed in a mixture of 1.5% glutaraldehyde and 1% formaldehyde in 0.1 M phosphate buffer (pH 7.4). For histology and transmission electron microscopy (TEM), these samples were processed according to the procedure of Lemaire et al. (1992) except that Spurr resin was used instead of Epon as the embedding material. A Hitachi H-6000 electron microscope was used for TEM.

For subcellular fractionation, the excised liver was washed with 0.9% saline and homogenized in a solution containing 0.2 M sucrose, 10 mM EGTA, 1 mM dithioerythritol and 25 mM HEPES buffer (pH 7.4) with a Potter-Elvehjem type glass homogenizer. The homogenate was filtered and subjected to differential centrifugation at 700 x *g* (10 min; pellet discarded), 10,000 x *g* (20 min; mitochondrial pellet), and 220,000 x *g* (45 min; microsomal pellet). The pellets were washed once by suspending in either the homogenization medium (mitochondrial pellet) or 0.15 M KCl (microsomal pellet) and recentrifuged at their respective *g* and duration. All fractionation steps were carried out at 0-4°C.

The activity of each enzyme was measured with limiting concentrations of enzyme source under optimal assay conditions. Mitochondrial rates of succinate oxidation, respiratory control ratio (RCR), and coupled phosphorylation (ADP:0) were measured according to Eastabrook (1967). The rates were assayed at 25°C. The activities of aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin-0-deethylase (ECOD), and the content of P-450 in the microsomal fraction were assayed as described previously (Khan et al. 1989). These assays were carried out at 18°C. Student's t-test was used to compare biochemical differences between control and 3-MC treated fish.

RESULTS AND DISCUSSION

No clinical signs of stress or intoxication were observed in control and 3-MC treated fish. Histological examination of liver and kidney tissues by light microscopy failed to show any differences between control and 3-MC treated fish. TEM of kidney showed marked differences in the size of tubular epithelial cells and in the ultrastructure of mitochondria and endoplasmic reticulum (ER) of control and 3-MC treated fish. The epithelial cells were considerably larger (Fig. 1b) in the 3-MC treated fish than in the control fish. The mitochondria in the control fish were tubular and elongated with distinct lamellar cristae and had an electron dense intramitochondrial matrix (Fig. 1a); however, many of these organelles in the 3-MC treated fish were oval or round with transverse and

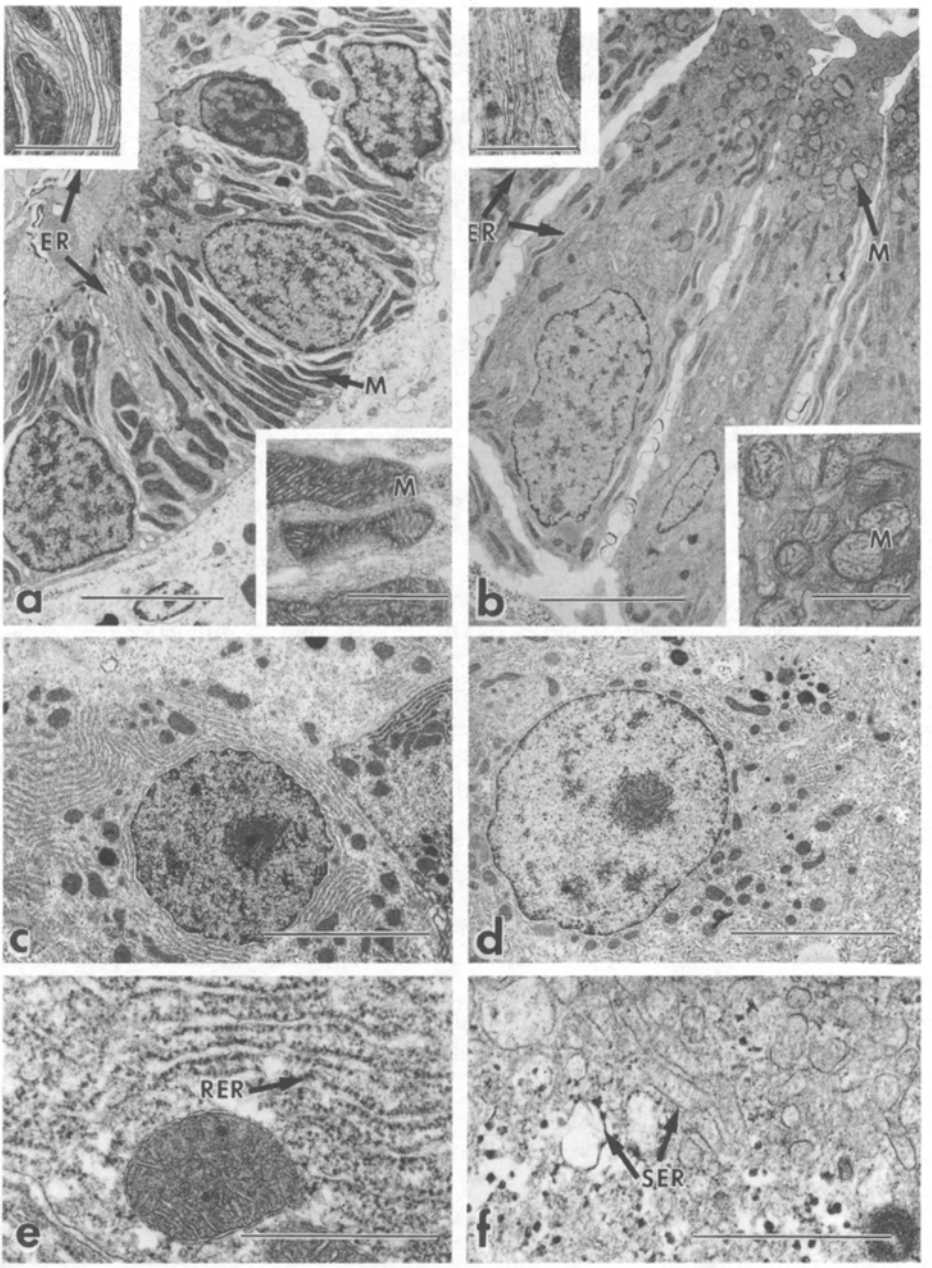

Figure 1. TEM of kidney in control (a) and 3-MC treated (b) rainbow trout, (bar = 5 μm); bottom insets show large elongated (a) and rounded (b) mitochondria [M], (inset bar = 1 μm). TEM of liver in control (c) and 3-MC treated (d) rainbow trout, (bar = 5 μm); higher magnification (bar = 1 μm) shows parallel arrays of RER in control (e) and increased SER in 3-MC treated (f) fish.

disorganized cristae and a flocculent intramitochondrial matrix (Fig. 1b, inset). These intramitochondrial changes are generally observed in degenerative mitochondria (Pritchard and Miller 1980). In addition, the tubular mitochondria in the 3-MC treated fish appeared compressed and smaller in size than those in the control fish. Such changes in mitochondrial volume indicate the ability of these cellular organelles to swell or shrink depending on the physiological and metabolic demands in the cytoplasm (Bereiter-Hahn and Vöth 1994). The ER in 3-MC treated fish was somewhat more abundant than in the control fish. These changes in kidney intramitochondrial and ER ultrastructure reflect the sensitivity of these organelles to PAH or PAH metabolites. Alterations in key biochemical processes can be responsible for such structural changes. For example, in mitochondria, the uncoupling of oxidative phosphorylation often leads to volume changes and related structural effects (Lehninger 1970). Such ultrastructural alterations in kidney tubular epithelial cells have not been reported previously for any PAH in rainbow trout. In teleost kidney, the swelling of mitochondria and ER was reported to represent early stages in the pathogenesis of toxic insult (Pritchard and Miller 1980).

In the liver, TEM of hepatocytes showed marked ultrastructural differences between the ER of control and 3-MC treated fish, but the mitochondria in these cells were not different (Fig. 1c,d). Treatment with 3-MC resulted in: (i) a decrease in rough endoplasmic reticulum (RER) and loss of structural organization (e.g. lack of characteristic arrays), (ii) an increase, and vesiculation of smooth endoplasmic reticulum (SER) (Fig. 1e vs Fig. 1f), and (iii) aggregation of free ribosomes. Such ultrastructural changes in hepatocyte ER also have been reported in liver of sea bass after a short-term treatment with another PAH, benzo(a)pyrene (Lemaire et al. 1992).

The effect of 3-MC treatment was also assessed on key metabolic indicators in mitochondria and ER (microsomes) of liver. The results in Table 1 showed that 3-MC treatment caused no deleterious effects on the biochemical characteristics of mitochondria as evidenced by: (i) no change in the rate of succinate oxidation under both phosphorylating (State 3) and nonphosphorylating (State 4) conditions, and (ii) good ADP:0 and RCR ratios. The lack of impairment of mitochondrial respiratory and bioenergetic metabolic function correlates well with the observed structural integrity of liver mitochondria in 3-MC treated fish.

Treatment of rainbow trout with 3-MC caused a marked and characteristic induction of P-450 and MFO (AHH, ECOD) activities in the liver (Table 1). The increase in P-450 was also accompanied by a shift in the spectral peak from 450 nm to 448 nm. Similar changes in P-450 level and associated MFO activities have been reported in other fish exposed to PAH, petroleum hydrocarbons and other lipophilic chemicals (Payne et al. 1987; James et al. 1988). The marked increase in AHH and ECOD activity in 3-MC treated fish is consistent with the observed increase in SER. The SER is particularly rich in MFO activities

Table 1. Effects of 3-MC treatment on biochemical parameters of liver mitochondrial and microsomal fractions of rainbow trout (means ± S.D., n=4)

Biochemical Parameters	Control	3-MC
Mitochondrial		
Succinate oxidation[a]		
(State 3)	22.3 ± 6.9	25.0 ± 4.5
(State 4)	6.5 ± 1.1	6.0 ± 0.9
RCR	3.4 ± 0.8	4.3 ± 1.0
ADP:0	1.9 ± 0.1	1.9 ± 0.1
Microsomal		
AHH[b]	1.7 ± 0.5	52.4 ± 22.9[e]
ECOD[c]	5.6 ± 0.7	81.6 ± 16.8[e]
P-450[d]	167 ± 48	278 ± 8[e]

[a] nmol oxygen uptake min^{-1} mg $protein^{-1}$
[b] pmol 3-hydroxybenzo(a)pyrene formed min^{-1} mg $protein^{-1}$
[c] pmol 7-hydroxycoumarin formed min^{-1} mg $protein^{-1}$
[d] pmol mg $protein^{-1}$
[e] values are significantly different from the control values ($p < 0.001$).

expressed by isoform P-450 1A1 (e.g. AHH). As demonstrated in this study, an increase in hepatocyte SER provides a good morphological indication of exposure to lipophilic PAH contaminants. This type of biochemical and structural alteration would potentiate the metabolism and removal of lipophilic xenobiotics from the liver. The nature of the metabolites formed would depend on the relative composition of P-450 isoform(s) induced in the SER. In this regard, it is interesting to note that benzo(a)pyrene biotransformation in liver of various fish species produced metabolites quite similar to those produced in rodent species, although the amounts of metabolites formed were different (Varanasi et al. 1989).

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