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Expression of Cytochrome P4501A (CYP1A) in Fish Gill (*Heteropneustes fossilis*) on Exposure to Aqueous Benzo[*a*] Pyrene

M.V. Radhakrishnan

Department of Zoology, Annamalai University,
Annamalainagar-608 002, Tamilnadu, India

Abstract: The freshwater catfish *Heteropneustes fossilis* were exposed for 45 days to environmentally relevant concentrations of aqueous (10 µg/liter) benzo[*a*]pyrene (BP) in static renewal aquaria. BP-inducible cytochrome P4501A (CYP1A) in gill was evaluated after 45 days exposure by immunohistochemistry in longitudinal histologic sections. Exposure to aqueous BP resulted in high levels of CYP1A-associated immunohistochemical staining in gill pillar cells and gill epithelia. The results not only give the design of more environmentally valid exposure protocols, but will provide useful information on bioavailability, fate and cycling of contaminants (polycyclic aromatic hydrocarbons and other compounds) in aquatic ecosystems.

Key words: Cytochrome P4501A · Gill · *Heteropneustes fossilis*

INTRODUCTION

Aquatic organisms may expose to organic toxicants during contact with contaminated food, water and sediment [1-5]. Though gill is considered to be the major route of toxicant uptake, the relative importance of each to overall exposure remains poorly understood. This information is needed because toxicity not only depends on dose and duration of exposure, but also on the route by which exposure occurs [6-11]. In vertebrates, exposure to diverse xenobiotics results in induction of one or more forms of enzymes involved in xenobiotic biotransformation [12,13]. Cytochrome P4501A (CYP1A) forms are the major oxidative enzymes induced in fish and other vertebrates by polycyclic aromatic hydrocarbons (PAHs) and polyhalogenated aromatic hydrocarbons (PHAHs) [14]. CYP1A responds to environmental levels of these compounds in a dose-dependent manner and is commonly used in field and laboratory studies to evaluate exposure and effects. Induction of CYP1A protein can be evaluated by immunohistochemical analysis of specific cells in histologic sections of organs [15 - 17]. CYP1A induction has been observed in tissues including those proximal to the environment such as gill [19]. In fish collected from contaminated environments, evidence for induction of CYP1A has been reported in multiple tissues and cells of histologic sections [16,20]. Moreover, immunohistochemical data are very useful in identifying cel ls and tissues capable of responding to xenobiotic exposure. Hence, in the present study attempts have been made to understand CYP1A expression in histologic sections of gill of the catfish *Heteropneustes fossilis* following exposure to 10 µg/liter benzo[*a*] pyrene, a common environmental polycyclic aromatic hydrocarbon.

MATERIALS AND METHODS

Irrespective of the sex, healthy specimens from *H. fossilis* of 36-38 g body weight and 18-20 cm length belonging to a single population were collected locally and were confined to large plastic aquaria bearing tap water for 30 days in the laboratory for acclimation.They were fed with mined goat liver on everyday (d) for 3 hrs (h) before the renewal of the medium. Water was renewed after every 24 h with routine cleaning of the aquaria leaving no faecal matter, dead fish (if any) or unconsumed food. Benzo [*a*] pyrene (BP) was chosen for this study because it is a common environmental contaminant of concern and a potent inducer of CYP1A. Fish were exposed to aqueous BP for 45 days and sampled at 5, 10, 20 and 45 days. Efforts were made to expose fish to the lowest concentrations of BP that produced a range of response in the tissue. Dose-response experiments

Corresponding Author: M.V. Radhakrishnan, Department of Zoology,Annamalai University, Annamalainagar-608 002, Tamilnadu, India. E-mail: radkisnan@yahoo.co.in

indicated that aqueous exposure to 10 µg/liter BP was sufficient to produce clear evidence of CYP1A induction in multiple tissues [21]. This value falls at the upper end of the estimated solubility of BP in seawater (5-10 µg/liter at 22°C) but is far lower than aqueous concentrations of CYP1A inducers used previously [18, 22]. Four groups of 10 fish each were exposed in static 10-liter aquaria amended with BP at concentrations of 10 µg BP/liter in 20µl acetone carrier/liter. Water and BP were completely replaced once a day. Parallel groups of control fish were kept. Control fish received acetone only. Preliminary experiments indicated no acetone effects on CYP1A.

After the expiry of 5, 10, 20 and 45 days of exposure five fish from the respective experimental and control fish were sacrificed using overdose with tricaine methane-sulfonate (MS-222). Second pair of gills were dissected out and fixed for 48 hr in Bouin's solution [23], washed overnight in running tap water and decalcified. To extract picric acid, tissues were washed in 50% ethanol saturated with lithium carbonate and subsequently dehydrated in a graded ethanol series and processed for paraffin histology by standard methods [23]. The immunohistochemistry protocol was a modification of the protocol for CYP1A detection in fish tissues developed by Cochran [24] using polyclonal antibodies (Ab) to cod CYP1A [25]. A Signet Level 1 Avidin-Biotin Complex (ABC) Detection Kit was used for localization of primary Ab and chromo-genesis. All incubations were performed in a humidified chamber. Longitudinal sections of gill was cut at 5 µm and adhered to gelatin-coated slides. Tissue sections were deparaffinized in xylene, hydrated to distilled water (dH_2O) in a graded ethanol series and washed in PBS. Endogenous peroxidase and biotin activity was quenched by application of 0.3% H_2O_2/40% methanol in PBS for 18 hr at 4°C. Nonspecific binding of Ab was blocked with 5% normal goat serum (NGS) in PBS for 40 min. Primary Ab (rabbit anti-cod CYP1A diluted 1:200 in 1% NGS/ PBS, 60 jul/slide) was applied directly over the NGS and incubated for 22 hr at 4°C. Biotinylated goat anti-rabbit IgG was added and incubated for 20 min at room temperature. The labeling reagent ultrastreptavidin (USA) was added and incubated for 20 min at room temperature. The substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB) was added and incubated for 5 min at room temperature. Slides were rinsed with dH_2O, dehydrated in a graded ethanol series and coverslipped. Parallel serial sections of each specimen were not immunostained, but stained with Harris' hematoxylin and eosin [23]. During evaluation of staining in histologic sections, the values assigned were of 0 (negative), + (mild), + + (moderate), + + + (strong). Negative values indicate that no visible stain was present, whereas high values imply a relatively high level of CYP1A present.

RESULTS AND DISCUSSION

CYP1A-associated staining in gill tissueare given in Table 1. Subjective qualitative evaluations focused on the gill pillar cells and gill epithelium. The control gill did not stain (Fig. 1). Following exposure of fish to aqueous BP, several cell types exhibited elevated staining relative to controls (Fig. 2). Gill pillar cells stained moderately at 5 days and high thereafter (Fig 3). In the present study, exposures resulted in high levels of CYP1A induction in gill pillar cells. Most importantly, the immuno- histochemical approach provides information on specific tissues and cells not available through Western blotting or assays of CYP1A-associated catalytic activity (e.g., EROD).Epithelial cells exhibiting CYP1A expression in the present study are similar to those reported previously following exposure to CYP1A inducers administered via ip injections or high-level aqueous exposures [15,16,18,22,25]. Thus, evidence for CYP1A-associated staining has been reported in gill pillar cells and gill epithelial cells. Strong induction in the endothelium of gill was observed in the present study. A similar induction in endothelium was previously reported in rats following exposure to cigarette smoke

Table 1: Showing CYP1A Staining*in the gill of the catfish to 10 µg/liter Benzo[a] Pyrene for 45 days

	Experimental				
Cell Types	Control	5d	10d	20d	45d
Epithelial Cells	0	+	+ +	+ + +	+ + +
Pillar Cells	0	+ +	+ +	+ + +	+ + +

*Staining intensity: 0 = negative; + = mild; + + = moderate; + + + = strong.

Fig. 1: Control gill showing negative staining of CYP1Ain gill lamellae X 788

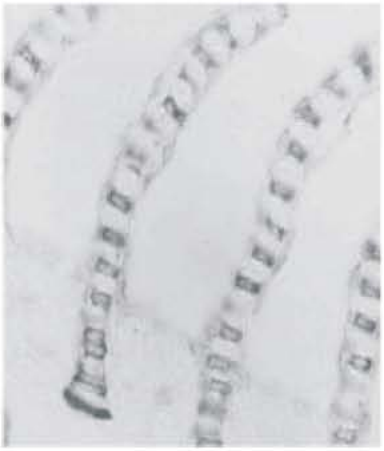

Fig. 2: Showing moderate staining of CYP1A in gill lamellae after 10days exposure X 788

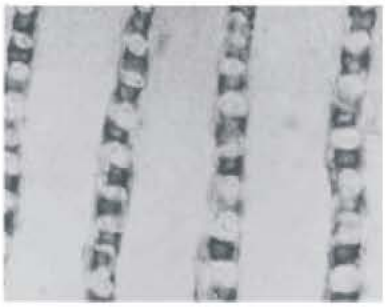

Fig. 3: Showing strong staining of CYP1A in gill lamellae after 45days exposure X 788

[26], as toxic metabolites of PAH produced by endothelial CYP1A could contribute to endothelial cell injury in exposed animals [14]. Alternatively, induction in these cells may serve as a defense for underlying cells. Studies with fish collected from PAH-contaminated sites suggested that tissue-specific patterns of CYP1A expression could contribute to understand exposure mechanisms in wild populations of fish [27]. However, development of an understanding of the numerous factors influencing the uptake and effects of toxicants in complex ecosystems is an extremely difficult and tedious process [28, 29]. Controversy over dominant routes of uptake of lipophilic toxicants by aquatic organisms is largely the result of difficulties in making and interpreting appropriate measurements. In the environment, routes of uptake are strongly influenced by the complexity of biogeochemical cycling of toxicants, species differences, temporal factors, trophic levels, feeding strategies, composition of food and sediment, lipid content of fish, sex, season, binding of toxicant to dissolved organic matter in water, complex mixtures of toxicants and multiple synergistic and antagonistic effects. The complexity of environmental factors which may influence the use of CYP1A immunohistochemistry in evaluation of exposure routes should not be viewed as a disadvantage. Rather, this type of approach could shed light on the influence that many of these factors have on bioavailability and cycling of toxicants in the ecosystem. The results of the study clearly shows not only be used in the design of more environmentally valid exposure protocols, but will provide useful information on bioavailability, fate and cycling of contaminants in aquatic ecosystems.

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