

# PROD and BROD Modulation in Nile Tilapia after Exposure to 17 $\beta$ -estradiol

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**Abstract:** The activities of 7-ethoxyresorufin-*O*-deethylase (EROD), 7-benzyloxyresorufin-*O*-debenzylase (BROD), 7-pentoxoresorufin-*O*-deethylase (PROD), and glutathione S-transferase (GST) were measured in Nile tilapias exposed for 7 days of 5 and 15  $\mu\text{g/L}$  17  $\beta$ -estradiol. EROD and GST activities were unchanged. PROD activity increased in animals exposed to the higher dose of the hormone, while BROD was increased after 7 exposure days to both doses of the compound. These results indicate the usefulness of these enzymes as biomarkers for 17  $\beta$ -estradiol exposure.

**Key words:** EROD, PROD, BROD, 17  $\beta$ -estradiol, biomarker, Nile tilapia.

## 1. Introduction

Endocrine disruptors (EDs) can be defined as exogenous agents that interfere with the synthesis, secretion, transport, reception, action or the elimination of endogenous hormones in organisms [1]. Among the most important endocrinal disrupters found in aquatic environments are natural and synthetic estrogens, because they are extremely active at very low concentrations and are related to the etiology of numerous cancers [2, 3]. Natural estrogens like 17  $\beta$ -estradiol, estriol, estrone and synthetic estrogens, developed for medical purposes [4], are among the most important concern, due to their high potency and the continuous use, with an increasing input in the environment. A significant amount of estrogens are excreted by humans in domestic wastes, being the human and animal excretion the main source of EDs in the aquatic environment [2, 3, 5].

The simple detection of EDs in water is not a simple approach, since exhaustive extraction procedures and expensive methods are sometimes needed [6]. Alternatively, the measurement of biochemical biomarkers can sometimes elicit the exposure of aquatic animals to EDs without the need of sophisticated chemical analyses.

One of the most common approaches for the evaluation of EDs effects in aquatic animals is the analysis of vitellogenin, precursors of the egg-yolk proteins [7]. Vitellogenin synthesis occurs only in mature females in response to endogenous estrogens [8], but in males, the vitellogenin genes becomes active in the presence of xenoestrogens [9], therefore being excellent indicator of exposure to EDs [10]. Among vitellogenin measurement, the search for alternative biomarkers for ED exposure in the aquatic environment could improve the options for aquatic biomonitoring studies.

The aim of this work was to investigate how the activities of some cytochrome P450 isoforms from

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liver of male Nile tilapia (*Oreochromis niloticus*) respond to low concentrations (5 and 15  $\mu\text{g/L}$ ) of 17  $\beta$ -estradiol (E2) in water after seven days of exposure. Despite this hormone is usually found at ng/L level in most studies in the literature, it was found at 3 to 5  $\mu\text{g/L}$  in the water resource of Campinas city (São Paulo, Brazil), thus justifying the concentrations tested in the present work [3].

## 2. Methodology

### 2.1 Exposure to E2

Twenty-seven male fish were separated into nine aquarium (3 fish per aquarium) containing 115 L of filtered, dechlorinated and aerated water. The fish remained at this condition for one week, then the hormone E2 was added to six of the nine aquariums at 5  $\mu\text{g/L}$  (3 aquariums) and 15  $\mu\text{g/L}$  (3 aquariums), composing three replicates for each group (N = 9): control, exposed to 5  $\mu\text{g/L}$  and exposed to 15  $\mu\text{g/L}$ . After seven days of exposure to the hormone, all fish were killed and had their gills and liver collected for enzymatic analyses. The average length and weight of the animals used in the experiment are presented in Table 1. Tilapia is generally considered adult by aquacultors after surpassing 30 g [11]. As the fish used in our experiments were among 190 g, they were considered all mature fish.

### 2.2 Biochemical Analyses

In liver, the activities of 7-ethoxyresorufin-*O*-deethylase (EROD), 7-benzoyloxyresorufin-*O*-debenzylase (BROD), 7-penthoxyresorufin-*O*-deethylase (PROD) and glutathione *S*-transferase (GST) were evaluated, and in the gill only GST was measured. The tissues were weighed and homogenized with 5 volumes of buffer

**Table 1 Values of length and weight (media  $\pm$  standard deviation) of tilapias used in the exposure experiment.**

	17 $\beta$ -estradiol (E2) ( $\mu\text{g/L}$ )		
	0	5	15
Length (cm)	15.0 $\pm$ 1.1	14.6 $\pm$ 1.3	13.9 $\pm$ 1.5
Weight (g)	193.3 $\pm$ 48.8	191.7 $\pm$ 47.8	193.8 $\pm$ 43.5

(Tris 50 mM, 0.15 M KCl, pH 7.4), and centrifuged at 10,000 g for 20 min at 4  $^{\circ}\text{C}$ . The supernatant fraction was centrifuged at 55,000 g for 60 min at 4  $^{\circ}\text{C}$ , in order to obtain the cytosolic and the microsomal fraction. The activity of GST was measured in the cytosolic fraction, while the activities of EROD, PROD and BROD were measured in the microsomal fraction.

The attribution of EROD, PROD and BROD to specific phase I biotransformation enzymes is controversial in the literature. The activities of EROD and PROD were generally referred to CYP1A and CYP2B, respectively, in fish and mammals [12]. In mammals BROD activity showed broader substrate specificity and was a known marker for CYPs 1A, 2B and 3A [13]. The activities of these CYP subfamilies were measured by the fluorimetric method described by Burke and Mayer [14], with some modifications. The substrates 7-ethoxyresorufin, 7-benzoyloxyresorufin and 7-penthoxyresorufin were used in the assay of EROD, BROD and PROD, respectively, at a final concentration of 5  $\mu\text{M}$ , and the assay was recorded (excitation 535 nm, emission 590 nm) during three minutes in the presence of 20  $\mu\text{M}$  of NADPH. The activity of GST was determined by the increasing in absorbance at 340 nm, using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates, according to Keen et al. [15]. The total protein content was determined by method of Bradford [16].

### 2.3 Statistical Analyses

Control and treated animals were statistically compared by the Student *t* test, by means of the Microcal Origin 6.0 (Northampton, MA, USA), and just  $P < 0.05$  was accepted as significant.

## 3. Results

No mortality was observed along the experiment. The activities of EROD and GST were unchanged comparing control and treated groups (Fig. 1). On the other hand, the activity of BROD was increased after seven exposure days to 5  $\mu\text{g/L}$  and 15  $\mu\text{g/L}$  of E2.

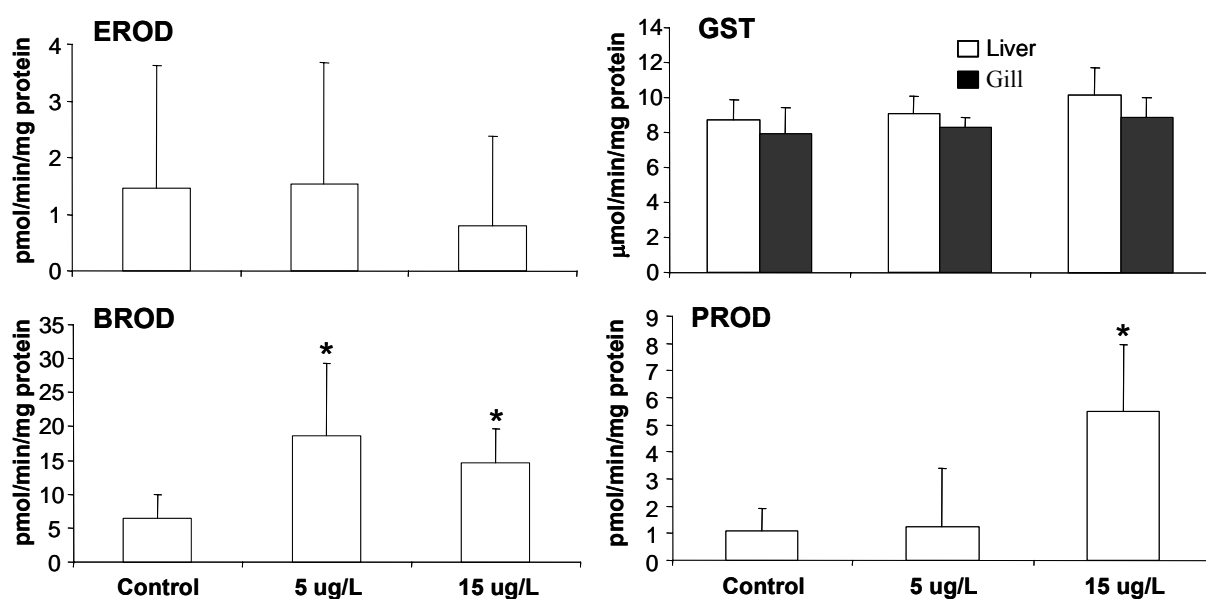


Fig. 1 Activities of BROD, PROD and EROD in the liver and of GST in the liver and gills of Nile tilapias exposed for 7 days to 5 and 15  $\mu\text{g/L}$  of 17  $\beta$ -estradiol. \*indicates statistical differences between related to control value ( $P < 0.05$ ).

PROD activity was increased only in those animals exposed to the higher E2 dose.

#### 4. Discussion

The present study preliminarily determined the responsiveness of GST, EROD, BROD and PROD activities to E2 in Nile tilapia. A number of reports have indicated that the suppression of the hepatic CYP1A and associated EROD activity occur in various male or female fish after experimental treatment with E2 or other xenoestrogens [17-20], although investigations into the effects of this hormone on other CYP isoforms in fish are scarce. Although not statistically different, we observed a trend of EROD activity inhibition in fish exposed to higher E2 dose.

EROD activity is generally related to CYP1A family, which are most related to polyaromatic hydrocarbons and polychlorinated biphenyls exposure, being not related to estrogen metabolism. Indeed, according to Matozzo et al. [7], most of hormones including E2 were conjugated with glucuronides before their excretion, thus GST could not be the main enzyme activated for E2 conjugation. Indeed, Hughes and Gallager [21] demonstrated that exposure of largemouth bass (*Micropterus salmoides*) to E2 (2 mg/kg) did not

affect steady-state GST-A mRNA expression in liver, despite GST activity increased after 2 days of exposure to E2, using CDNB as substrate. It could be possible that GST increased as well in tilapias from our experiments after 2 days of exposure, but fish were collected only after 7 days of exposure, and this remains to be confirmed.

On the other hand, the activities of BROD and PROD were very sensitive to this hormone, being increased after seven days of exposure to 5 (BROD) and 15  $\mu\text{g/L}$  (BROD and PROD) of E2. This strongly suggests the involvement of these enzymes in metabolism of the hormone. It is interesting the fact that these two enzymes increased strongly after exposure to very low levels of the contaminant, indicating their high sensibility to this hormone and their potential use as exposure biomarkers for the presence of E2 at  $\mu\text{g/L}$  levels in water. Regardless of CYP isoform specificity of the substrate used for the activity of P450 in exposed fish, the present study demonstrated that E2 exposure modulated the activity levels of these putatively different CYP subfamilies. Further studies should be done with less E2 concentration and longer exposure periods, since most of environmental studies had detected this hormone at

ng/L levels in the aquatic environment [22].

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