Pollution, 2(1): 77-82 , Winter 2016
DOI: 10.7508/pj.2016.01.008
Print ISSN 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir Email: jpoll@ut.ac.ir

Alterations in the Plasma Thyroid and Cortisol Hormones in Yellowfin Sea bream, *Acanthopagrus latus*, following exposure to Benzo(α)Pyrene

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Received: 9 Sep. 2015 Accepted: 18 Sep. 2015

ABSTRACT: The goal of this research is to study the effect of benzo-alpha-pyrene (BαP) as a pollutant on the plasma levels of cortisol, thyroxin (T4), and triiodothyronine (T3) hormones, and the T3/T4 ratio in the yellowfin sea bream, *Acanthopagrus latus*. The BαP (50 mg kg^{-1}) in vegetable oil was peritoneally injected. Blood samples were obtained from the treated and control groups after 3 and 72 hr, respectively. The amounts of cortisol, T3, and T4 were measured using the ELISA techniques. The results showed that during both the 3 and 72 hr BαP exposures, the T4 hormone levels significantly decreased, although the cortisol levels increased ($P<0.05$). However, the T3 hormone levels and T3/T4 ratios compared with their control groups showed a significant difference just after 72 hr ($P<0.05$). The disruptive effects of the BαP exposure on T4 was stronger than that on the T3, being more evident in long-term stress. Thus, the BαP exerts a significant effect on the thyroid endocrine system and consequently on fish metabolism and growth.

Keywords: BαP, cortisol, sea bream, thyroxin, triiodothyronine

INTRODUCTION

Polyaromatic hydrocarbons (PAH) such as benzo (α) pyrene (BαP) are found in marine environments polluted with petroleum products (Hylland, 2006). These compounds affect the physiology of the marine animals. PAH may activate the aryl hydrocarbon receptors (AhR) and stimulate the CYP1A cytochrome (Aluru *et al*., 2005). The cytochrome is part of an enzymatic system and produces more reactive intermediates which interfere with the production, secretion, and cycles of hormones such as cortisol and the thyroid hormones (Teles *et al*., 2005). These hormones play important roles in the responses of fish to stress.

The secretion of the thyroid hormones, including T3 and T4, is under the control of the hypothalamus-pituitary-thyroid (HPT) axis, whereas the cortisol secretion is controlled by the hypothalamus-pituitary-interrenal (HPI) axis (Bernier and Peter, 2001; Power *et al*., 2001). Therefore, the HPT and HPI axes play pivotal roles in most homeostatic mechanisms in fish. Thyroid hormones play a crucial role in regulating growth through their effect on the hydromineral balance of the body fluids (Van Anholt *et al*., 2003). Cortisol, on the other hand, is important in regulating the metabolic energy, stress responses and immune system (Belanger *et*

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al., 2001). Thyroid hormones and cortisol influence the PAH metabolism (Hontela *et al*., 1995). The plasma levels of T4, T3, and the T3/T4 ratio are used as the index for estimating the status of metabolism and growth, such as the synthesis rates for the proteins, and the oxygen consumed by the tissues (Fontaínhas-Fernandes *et al*., 2000; Gad and Saad, 2008).

In teleosts, the thyroid hormones (TH) are significantly involved in many physiological processes such as osmoregulation, growth, metamorphosis and reproduction. Releasing the thyroid-stimulating hormones from the pituitary can activate the thyroid glands to secrete T4. More than 95% of the thyroid hormones occur as T4, which can change into the biologically active T3 form (Power *et al*., 2001). Thyroid hormones affect the dynamics of the growth hormones and insulin-like growth factor 1 (IGF-I) during embryonic evolution (Kobuke *et al*., 1987) and maturation (Schmid *et al*., 2003).

The HPT axis is quite sensitive to the marine petroleum pollutants and is highly vulnerable. Many studies show that the xenobiotics can disrupt the physiological processes under the control of this axis through their agonistic and antagonistic effects (Leatherland, 2000).

The PAH are considered significant pollutants of the marine ecosystems entering the seas due to human activities like petroleum extraction, industrial and domestic waste or even naturally from decomposing organic matter. The BαP is a high molecular weight PAH. When this compound is decomposed within the cells, it has the immense potential to produce free radicals and intermediate carcinogenic compounds (Meador *et al*., 2006). These intermediate compounds can cause disturbances in the endocrine system, especially in the cycle of the thyroid hormones (Brown *et al*., 2004). Organic pollutants, such as PAH, can disturb the normal hormone concentrations, and consequently, affect other parts of the endocrine system and metabolism. These changes occur by influencing the hypothalamus-pituitary secretions, changing the production and release of the cortisol, T3, and T4 (Capen, 1997), and disrupting the hepatic 5-monodeiodinase (Hontela *et al*., 1995).

The aim of this work was to study the effect of the BαP as an important marine pollutant on the plasma levels of the cortisol, T3, and T4 hormones, and the T3/T4 ratio after 3 and 72 hr of exposure, with respect to the vital part these hormones play in the metabolism and growth of fish.

MATERIAL AND METHODS

First, 28 yellowfin sea bream, *Acanthopagrus latus* (average weight 156 ± 7g), were caught by trolling in the Musa estuary, in the north of the Persian Gulf, and transferred to the Marine Fish Research Center in Imam Khomeini Port (Khuzestan, Iran). To adapt to the new conditions of light and temperature, they were maintained in tanks (300 l) for two weeks, and fed daily at 1% of body mass with commercial dry pellets (Dibaq-Diprotg S.A., Segovia, Spain). They were starved for 24 hr prior to BαP exposure.

Two experiments were performed to study the acute and potentially chronic effects of BαP. The selection of the BαP dose (50mg kg^{-1}) for injection and implantation were determined from the earlier studies done to determine the time and concentration effects of the petroleum hydrocarbons and their derivatives on some tissues and cellular processes (Pacheco and Santos, 1998; Tintos *et al*., 2008; Wilson *et al*., 1998). In the first experiment, 14 fish were placed in two separate tanks, control (n=7), and treatment group (n=7). Prior to the injection, the fish were anesthetized with 2-phenoxyethanol solution (0.2%) (P1126, Aldrich) and weighed. The treatment group was administered BαP

(50mg kg^{-1}) in sunflower oil (2µl g^{-1}) via intraperitoneal injection. The control group received only the sunflower oil (2µl g^{-1}). Blood samples (2cc) were obtained 3 hr post injection.

The second experiment (n=14) was similar to the first one, except that coconut oil (10µl g^{-1}) (for the slow-release of BαP into the blood) was used in the treatment (n=7) and control (n=7) group instead of sunflower oil and the blood samples were obtained 72 hr post implantation.

After anesthetizing the fish, blood sampling was done from their caudal veins using a heparinized syringe. The blood samples were kept on ice until all the samples were obtained. Then, after centrifuging (Hettich-D7200, Tuttlingen, Germany) the blood at 6000 rpm for 6 min, they were rapidly frozen in liquid nitrogen. They were maintained at -80˚C until the hormonal assays were performed. The amounts of cortisol, T3 and T4 were measured carefully using the ELISA techniques with the commercial kits (Radim, Rome, Italy) per the manufacturer's instructions. An ELISA reader 1 (Sirio S, SEAC Radim, Rome, Italy) was used to measure the amounts of cortisol, T3, and T4.

The average amounts of cortisol, T3, T4, and the T3/T4 ratio in the control and treatment groups were compared using the t-Test. Significant differences required an α level of 0.05. The Sigma plot ver. 11 Software (Systat Software, Inc., CA, USA) was used for analyzing the data and drawing the diagrams.

RESULTS

No abnormal behaviors were observed with respect to the swimming, movements, and mortality rates among the fish. The results revealed that the plasma cortisol levels during the short-term (3 hr) and the long-term (72 hr) treatments showed a significant difference compared with the control group ($P<0.05$) (Fig. 1). The BαP exposure stress on the T3 levels of plasma did not result in any significant differences between the control and treatment groups 3 hr post the injection ($P>0.05$). However, with prolonged stress (72 hr), the T3 hormone levels showed a significant decrease between the control and treatment groups ($P<0.05$) (Fig. 1a). In both the 3 and 72 hr BαP exposures, the T4 hormone levels showed significant decreases ($P<0.05$) (Fig. 1b).

During the acute stress, no significant changes were observed in the T3/T4 ratio. However, during the prolonged stress, the T3/T4 ratio showed a significant decrease. ($P<0.05$) (Fig. 1.d).

DISCUSSION

The BαP treatment induced an increase in the levels of the plasma cortisol during the 3 and 72 hr BαP exposures. The results were similar to those obtained from the research of Thomas *et al*., (1995) on *Mugil cephalus*, Kennedy and Farrell (2005) on *Clupea harengus*, and Tintos *et al*., (2008) on *Oncorhynchus mykiss* about the effect of PAH on the plasma cortisol levels. As the BαP enters the body of a fish, it activates the AhRs. Although the mechanism of these receptors and the location of their effect is still unclear, it is suggested that their activity induces disorders in the HPI axis and raises the cortisol levels by increasing the corticotrophin releasing factor (CRF) gene expressions in the hypothalamus, resulting in an increase in the CRF factor and ACTH hormone (Vijayavel *et al*., 2006). Although Teles *et al*. (2005) reported that exposure to the PAH was the reason for the drop in the plasma cortisol levels in their studies, they reported that the inefficiency of the HPI axis or the loss of mitochondrial cortisol-synthesizing enzymes were the main factors.

The plasma levels of T4 showed a significant decrease 3 hr post BαP injection. However, no change was observed in the T3

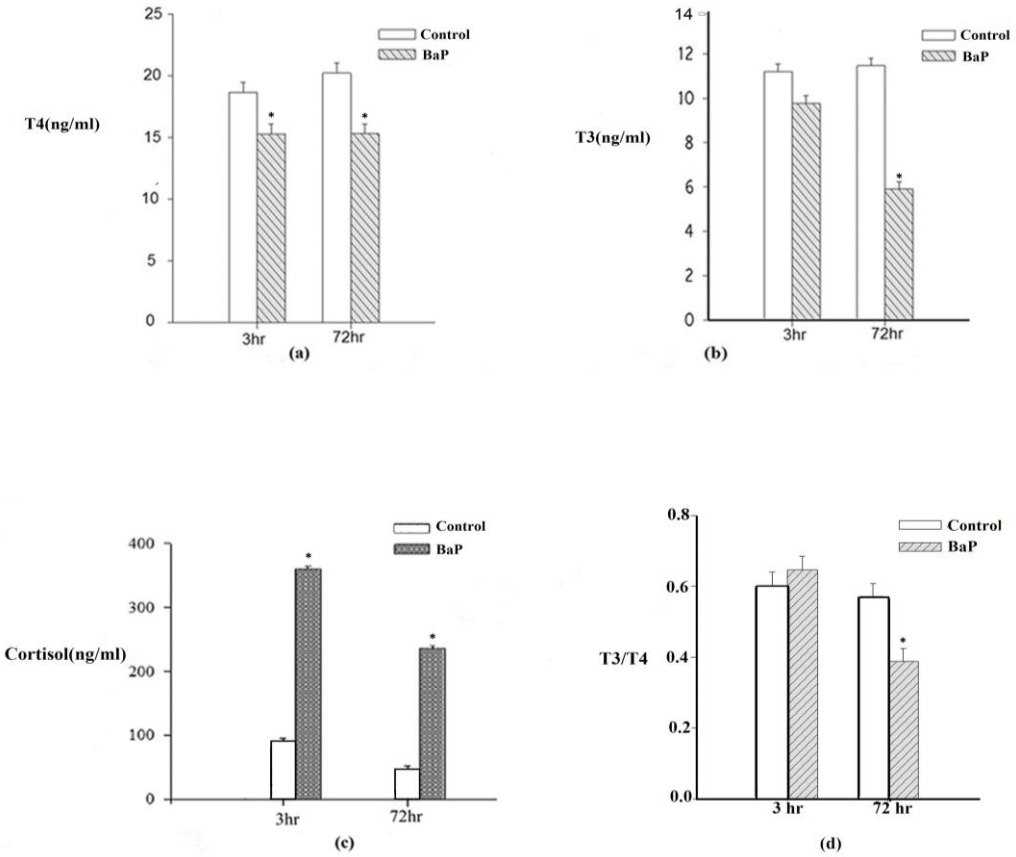

Fig. 1. Effect of 3 and 72 hr BαP treatments on the responses of the plasma T4(a), T3(b), cortisol(c) levels and T3/T4 ratio(d). The star symbol indicates significant difference between the treatments and control group.

levels and the T3/T4 ratio. After prolonged stress, there was a decrease in the plasma levels of the thyroid hormones and the T3/T4 ratio. A few studies have been done earlier on the effect of the PAH on the thyroid hormones. Rolland (2000) observed that PAH induced a decrease in the thyroid hormones and changes in the morphology of the thyroid gland. Similar results were reported by Stephens *et al*., (1997) in salmon, and Teles *et al*., (2005) in the European eel. Moreover, lowered levels of the thyroid hormones and changes in the T3/T4 ratio were reported in fish that were exposed to crude oil (Hontela, 1998). Alterations in the thyroid hormones and T3/T4 ratio can be used as a biomarker for the pollutants (Eales *et al*., 1998). However, the mechanism of the effect on the thyroid hormones by the xenobiotics is quite complicated. The PAH can cause disturbances at different stages of gene expression, synthesis, release, secretion, and transformation of the thyroid hormones. The BαP activates the aryl hydrocarbon receptors (AhRs) when it enters the body (Nacci *et al*., 2002). The activation of these receptors can change the expression patterns of those genes that code for the thyroid hormones, and their related genes in the pituitary, hypothalamus or thyroid tissues (Gauger *et al*., 2007). Any disruption in the expression of such genes can result in the decrease and inefficiency of the synthesis and secretion of the thyroid hormones. The PAH can induce the disruption of the synthesis pathways of the thyroid hormones by changing the thyroglobulin iodination or T4 deiodination which converts it into T3 (Brown *et al*., 2004). By binding to the homodimer and heterodimer receptors of the thyroid, such pollutants can induce

changes in the gene expression and the activity of the enzymes involved in the thyroid hormone cycles such as phosphoenolpyruvate carboxykinase and 5'-monodeiodinase, decreasing the plasma levels of the hormones (Jakobs *et al*., 1997; Park *et al*., 1995).

Some xenobiotics can compete with the T4 carrier proteins in the plasma (TTR proteins) and bind with them. As a result they stop the T4 from binding to such proteins and prevent their transport in the plasma (Purkey *et al*., 2004; Ucán-Marín *et al*., 2009). Binding the BaP with the TTR proteins during acute stress can induce changes in the T4 levels, consequently changing the T3 levels with prolonged stress. These changes commonly send signals to the whole HPT axis, and disturb the thyroid hormone cycles.

CONCLUSION

Generally, the results confirm that the acute and prolonged stresses of BαP alter the metabolic cycles of the thyroid hormones. The disruptive effects of BαP exposure on the T4 is stronger than that on the T3, and is more evident during long-term stress. According to the importance of the thyroid hormones in the metabolism and homeostasis in fish, and their high sensitivity to pollutants, any alteration in the plasma levels of these hormones and T3/T4 ratio induced by BαP pollution can result in growth and behavioral changes.

ACKNOWLEDGMENTS

Our sincere thanks is due to Dr. Joe M. Regenstein from the Dept. of Food Science at Cornell University, (Ithaca, NY, USA) for his extensive editorial and scientific review of the manuscript. The authors would like to thank Mohammadreza Sahraeian and Hossein Pasha for their technical and scientific assistance, and Khorramshahr University of Marine Science and Technology for providing the laboratory and use of the instruments.

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