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Research Article

Changes in the Pituitary and hypothalamus Monoaminergic Neurotransmitters after Acute and Prolonged Stress Exposure to Benzo (α) Pyrene in *Acanthopagrus latus*

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Abstract

In this study, the effects of the Polycyclic Aromatic Hydrocarbon (PAH) Benzo (α) pyrene (BαP) exposure on the levels of serotonin (5-HT) and dopamine (DA) in the pituitary and hypothalamus of Yellowfin seabream, (*Acanthopagrus latus*) were examined. To assess the acute stress responses, vegetable oil (2 µl g-1) containing BαP (50 mg kg-1) was injected into the treatment group of fish (the vegetable oil alone was the control), and brain samples from different groups were collected 3 hr after injection. Base line group was not injected. To study the long-term stress, brains were collected from both injected groups after 72 hr. The amounts of serotonin, dopamine, and amine metabolites in the hypothalamus and pituitary were measured. Results showed that BαP influenced the serotonergic system more than the dopaminergic system for both acute and prolonged stress in both the hypothalamus and pituitary. Acute exposure to BαP induced significant decreases in DA and increases in DOPAC (3, 4-dihydroxyphenylacetic acid) in the pituitary ($P<0.05$). Major changes induced by both acute and prolonged exposure to BαP included significant decreases in 5-HT, increases in 5-HIAA (5-hydroxy-3-indoleacetic acid) and increases in the 5-HIAA/5-HT ratio ($P<0.05$). These exposures might affect the synthesis, storage, uptake/release, and degradation of the neurotransmitters in the hypothalamus and pituitary of sea bream, especially the pituitary gland.

Introduction

PAHs are one of the most important environmental pollutants, especially in oil extraction regions and may disrupt internal homeostasis. In the marine environment, its water-stability fractions contain Polyaromatic Hydrocarbons (PAH) such as benzo *(α)* pyrene (BαP) that could induce stress in fish. A primary respond to this stress is the release of neurotransmitters. Monoamine neurotransmitters are in dolamine, catecholamine, or imidazolamine. These compounds are responsible for cell signaling and neural controlling as well as having a role in endocrine and immune system function [1]. Monoaminergic systems in the nervous system have remained unchanged during the evolution of vertebrates [2]. These systems link behavioral and physiological functions. Hence, such pollutants as PAHs that affect neurotransmitters will induce alterations in the biological condition of individuals and populations [3,4]. Stress responses in fish result from stimulating the Hypothalamus-Pituitary-Interrenal (HPI) axis and may result in metabolic and physiologic changes in fish to help tolerate stressful conditions. Like all vertebrates, physiological responses to stress in fish are controlled by mechanisms in the brain. Some neurotransmitters such as serotonin (5-HT) and Dopamine (DA) have key role in this process. It has been shown that 5-HT secreted from raphe nuclei and the hypothalamus paraventricular in the brain of Arctic char stimulated the HPI axis during stress [5].

PAHs induce biological stress responses by activating Aryl hydrocarbon Receptors (AhRs). BαP, a PAH, is dissolved in brain tissues because of its lipophilic nature, and disturbs the function of the neurones. The specific responses during synthesis, secretion, storage, and reuptake of monoamines in different parts of the brain depend on the uptake level of the pollutant [6,7].

Adaptation to stress is important for survival and reproductive success in teleosts (bony fish). An assessment of the brain levels of neurotransmitters may help to understand physiological and behavioral responses after exposure to stressors. There had been limited research with fish on the effects of organic pollutants on brain levels of neurotransmitters as well as their impact on hormonal responses to stress conditions but it has been increased in recent years and shows its importance. The Persian Gulf is an important region for petroleum extraction and consequently polluted with PAHs. The present study was conducted to determine the effects of acute and chronic exposure to BαP on the levels of serotonin and dopamine in the pituitary and hypothalamus in the brains of yellow finseabream *(Acanthopagrus latus)*. Our hypothesis is that BαP disturbs the fish's neurotransmitters.

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Material and Methods

Experiments and samplings

Yellowfin seabream, were caught by trolling in Musa creek, which is located in the northern part of the Persian Gulf (Iran). The live fish were transferred (in water tank) to the Imam Khomeini Marine Fish Research Center (located on the beach). Seventy five fish (156±7g) were held in15 aerated tanks (300 l) for 2 wk to acclimate. Fish were fed (1% body weight) daily commercial dry pellets (Dibaq- Diprotg S.A., Segovia, Spain) until 24 hr before initiating the experiments.

Acute stress exposure to BαP

Forty five fish were divided into 3 groups (base line, control, and treatment) (n=15). The fish were injected and not exposed to BαP (because it is not dissolved in water easily and consequently takes long time to show its effect). Before BαP injections, fish were anesthetized with 0.2% phenoxy ethanol solution (S393975, Sigma), and weighed. Fish in the treatment group were in traperitoneally injected with sunflower oil (2μl g^{-1} body weight) containing BαP (50 mg kg^{-1}) while the control received the oil without BαP. To assess the effects of handling and injection stress on fish, the base line group (neither solvent nor BαP) was not injected. Sampling from all the groups was done 3 hr after the fish had been injected.

Long-term stress exposure to BαP

In the second experiment, 30 fish were divided into 2 groups (control and treatment) (n=15). After anesthetizing with phenoxy ethanol (0.2%), fish in the treatment group were in traperitoneally injected with coconut oil (46949: Sigma) (10 μl g^{-1}) (for slow-releasing of BαP into blood) containing BαP (50 mg kg^{-1}) while the control received just the oil. Fish were sampled 3 days after injection. The amount of BαP was chosen based on an assessment of previous studies that studied time and dose effects of PAHs and their derivatives on different tissues and cellular processes [6,8,9].

Processing the Fish

Fish were anesthetized using 2-phenoxyethanol (0.2%) and fish were beheaded. The brain and its different parts, including the pituitary and hypothalamus [10,11], were separated and weighted. All tissues were quickly frozen using liquid nitrogen and kept at -80 ^{0} C for no longer than one week.

Level of Neurotransmitters

The level of neurotransmitters (5-HT, DA, and their most important oxidized amines) were measured [12,7] using High Performance Liquid Chromatography (HPLC) (Waters 2695, Waters Associates, Jamaica, New York, USA) which was equipped with an electrochemical detector (Waters 2465) and automatic injection platform. The HPLC column used for separating the compounds was a 25 cm C18 with 4μm diameter (Waters Associates). The first electrode was set at +40 mV and the second at +340 mV (against an Ag/ AgCl reference electrode).

The liquid phase contained 63.9 mMNa H_2PO_4, 0.1 mM Na_2EDTA, 1.63 mMNa 1-octanesulfonate and 14.9% methanol. The pH was brought to 2.79 with acid orthophosphate.

Each brain tissue sample was separately homogenized using an ultrasonic homogenizer (150VT, BioLogics, Inc, Manassas, Virginia, USA). Then, the volume of mobile phase, which was 0.1 ml for pituitary and 0.5 ml for hypothalamus was added to the vial containing brain tissue (pituitary and hypothalamus separately), and then homogenized. These tissue extracts were centrifuged for 10 min at 16000 x g (Z300 non-refrigerated, Labnet International Inc., Edison, NJ, USA). The supernatant was removed. Mobile phase was added to the hypothalamus (2:1 v/v) supernatant but not the pituitary supernatant. These samples were injected into the HPLC. Volume of injection for each sample was 30 μl; run time was 15 min; and the isocratic flow rate was 1.1 ml/min at ambient temperature.

Statistical analysis

Levels of neurotransmitters were expressed as mean ± standard error. The standard deviations were calculated for 15 repeats of the experiment (n=15). A one-way ANOVA test was used to compare the mean concentrations of the neurotransmitters in the three groups for the first experiment and two groups for the second. The Student-Newman-Keuls test was done to evaluate multiple comparisons. The significance level was set at $P < 0.05$. The Sigma plot ver.11 software (Systat Software, Inc., CA, USA) was used for data analysis and diagramming.

Results

Changes in DA, DOPAC (3, 4-dihydroxyphenylacetic acid), and the ratio of DOPAC/DA

DA increased and DOPAC decreased significantly ($P < 0.05$) in the pituitary compared to both the base-line and control groups with acute stress. The DOPAC/DA ratio increased significantly after 3 hr in the pituitary ($P<0.05$). However, prolonged exposure to BαP did not induce significant differences in DOPAC, DA, and DOPAC/DA in the pituitary. There were no significant effects on dopaminergic system in the hypothalamus (Figure. 1).

Changes in 5-HT, 5-HIAA, and the ratio of 5-HIAA/5HT

BαP influenced the serotonergic system more than dopaminergic system with both acute and prolonged stress. Both acute and prolonged exposure to BαP had significant effects on the three components of the serotonergic system. Acute stress induced a

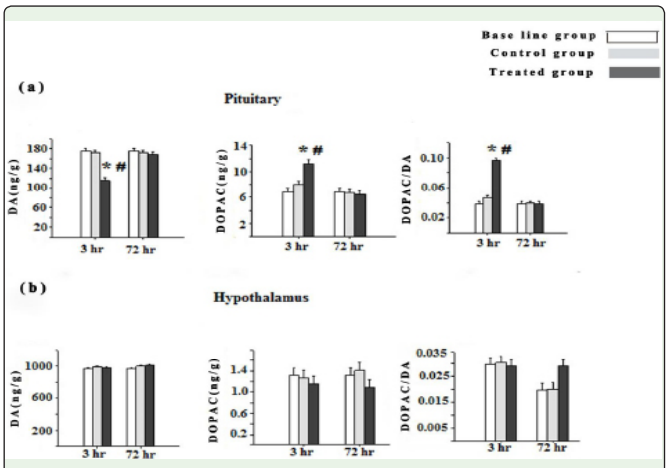

Figure 1: Changes in DA and DOPAC concentrations and the ratio of DOPAC/DA in pituitary (a) and hypothalamus (b) after acute (3hr) and prolonged (72hr) exposure to BαP (mean ± S.E.M.).

*: Significant difference from the base-line group
#: Significant difference from the control group

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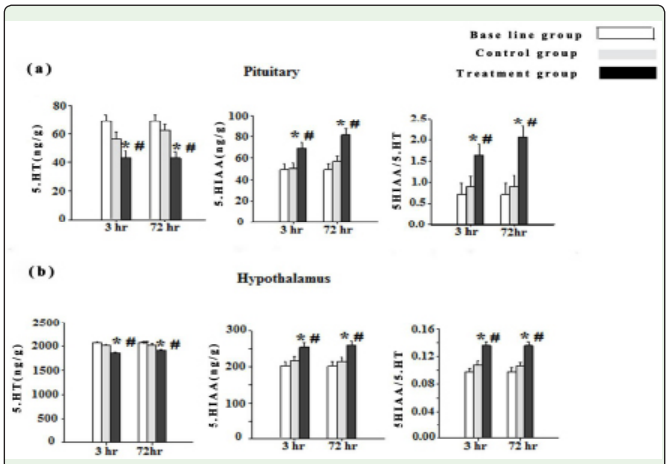

Figure 2: Changes in 5-HTconcentration and 5-HIAA and the ratio of 5-HIAA/5-HT in pituitary (a) and hypothalamus (b) after acute and prolonged exposure to BαP (mean ± S.E.M.).

*: Significant difference from the base-line group
#: Significant difference from the control group

significant reduction of 5-HT concentration in both the hypothalamus and pituitary compared to the base-line and control groups ($P<0.05$). The mean concentration of 5-HIAA (5-hydroxy-3-indoleacetic acid) and the ratio of 5-HIAA/5-HT increased significantly in both the hypothalamus and pituitary after acute and prolonged exposure to BαP ($P<0.05$) (Figure 2).

Discussion

During short-term stress, BαP induced wider effects on the 5-HT system compared to the DA system, and led to decreased 5HT and increased 5-HIAA in the hypothalamus and pituitary. To better describe the activity of neurotransmitters, the major metabolites ratio of a monoamine to the same monoamine is considered a more appropriate indicator compared to measurement of the absolute levels of monoamines. In fact, the concentration of a monoamine alone cannot express the neuron activity related to that neurotransmitter [13,14].

Increased serotonergic activity in acute stress was observed in both the hypothalamus and pituitary. Similar to the results of the current study, Gesto et al (2008) reported increased activity of 5-HT neurons in the brain of trout treated with BαP and β-naphtoflavone. In addition, Stephanou et al. (1998) observed increased activity of 5-HT neurons in the brains of mice 6 to 12 hr after treatment with BαP.

It seems that BαP prevents synthesis of 5-HT, and such an inhibition occurs more intensively with acute exposure in the hypothalamus [10,11]. BαP might have caused the reduction of 5-HT release from the synaptic terminals indirectly through reducing stimulatory inputs or increasing inhibitory inputs to serotonergic neurons. Khan and Thomas (2001) mentioned the dysfunction of the tryptophan hydroxyl laseenzyme, which is involved in the synthesis of 5-HT as one possible mechanism for a decrease in 5-HT.

Reduction in the 5-HT concentration during acute stress was associated with increased 5-HIAA content and 5-HIAA/5-HT ratio that suggests 5-HT consumption and increased activity of 5-HT neurons due to BαP chemical stress. Increases in 5-HIAA concentrations and indicators of 5-HT activity may be due to rising level of another amino acid synthesizing enzyme of 5-HT, that is tryptophan, since BαP can stimulate enzymes that are involved in the synthesis and metabolism of 5-HT [7,15].

During acute stress exposure to BαP, dopaminergic activity did not change much. Based on the results, the only change in these conditions was a decrease in the pituitary DA content with an increase in DOPAC concentration and their ratio. BαP probably caused the increased DA neuron activity and thereby increased the absorption and uptake rates of DA. In addition, BαP is likely to indirectly decrease DA release from the synapthic terminals through a decrease in stimulatory inputs or an increase in inhibitory inputs to the DA neurons, which is more strongly reflected in the pituitary.

BαP treatment caused the stimulation of monoamines activity in the brain, but the changes in the patterns were not identical for different tissues at different times. Activation of AhR receptors caused an increased consumption of oxygen, while high concentrations of unsaturated lipids and/or reduction of catalase enzyme activity caused a lowering of brain oxygen to the critical level where oxidative damages can be seen [16]. Oxidative damage can negatively interfere with the function of enzymes involved in the synthesis of catecho lamines and the immune system transporters.

The aminergic system of the hypothalamus and pituitary in fish are often the most sensitive sites responding to stress. Some of these neurotransmitters are essential for the coordination of social behaviors such as group responses [5,17], escape [18] and feeding [19]. The results suggest that BαP treatment, both short term and long-term, will cause disturbances in neuroendocrine responses in *Acanthopagruslatus* and reduce the fish's ability to cope with stress.

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