MODULATION OF GENE EXPRESSION IN GONAD AND LIVER OF MALE GOLDFISH EXPOSED TO BISPHENOL A

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Introduction:

Bisphenol A (BPA) is widely used for production of polymers such as polycarbonate [1, 2]. Its levels were reported between 0.02–21 ug/L in river water [1]. BPA is known as an estrogen mimicking compound; but there are a few evidence for its anti-androgenic activity depending on dose and period of exposure [3]. Adverse effects of BPA on intersex, vitellogenin (VTG) induction and decrease of androgens and sperm quality have been shown in some studies [1, 2, 3]. The main objectives of the present study was to investigate modulations of gene expression in gonad and liver of male goldfish exposed to environmentally relevant concentrations of BPA.

Methods:

Mature males of goldfish were exposed to BPA at 0.0, 0.2, 2, and 20 ug /L for 90 days during the spawning season. Samples of liver and gonad were collected at days 7, 15, 30, 60, and 90 after exposure. Expressions of ER subtypes, AR, StAR and CYP19Awere analysed in gonad. In liver, analysis of VTG, ER subtypes and AR mRNA expression was performed. Total RNA was extracted from each sample using TriZol Reagent (Invitrogen). cDNA was synthesized from total RNA using an oligo-d(T) anchor and M-MLV reverse transcriptase (Invitrogen) according to the manufacturer's protocol. Quantitative Real-time PCR (iCycler iQ Multicolour Real-time PCR Detection System, Bio-Rad Labratories, Inc) was used for evaluating gene expression level. Relative mRNA expression from the control was used in statistical analysis after testing homogeneity of variance and normality of data.

Results:

Relative ER-beta1 expression in gonad was significantly increased at 20 ug /L after 7 days, while its expression in liver was increased at 2 ug/L after 60 days. Relative ER-beta2 expression either in gonad or in liver was increased at 20 ug/L after 60 days. Relative ER-alpha was increased in liver or gonad at 2 or 20 ug/L after 60 days, but the difference was not significant. Significant increase of relative VTG mRNA expression in liver was observed at 2 ug/L after 60 days exposure. Relative AR mRNA expression in gonad showed significant decrease at 0.2 and 2 ug/L after 7 and 15 days exposure, but increased significantly at 2 ug/L after 90 days exposure. In liver, relative AR mRNA expression did not change within the exposure period. Relative StAR mRNA expression in gonad decreased at 0.2 and 2 ug/L after 90 days exposure. Significant increase of CYP19A was observed aft 20 ug/L after 60 days.

Discussion:

Our previous studies showed that BPA decreased androgen (T and 11-KT) production at low concentrations (0.5–1.5 ug/L) [4]. The observed decrease of androgen production might be corresponding to low cholesterol delivered into steroidogenesis pathway. Because, relative StAR mRNA expression, which deliver cholesterol to the inner mitochondrial membrane to be converted to pregnenolone via P450scc, decreased at low concentrations (0.2 and 2 ug/L) in the present study. The present study also showed that BPA at 0.2 and 2 ug/L decreased the AR mRNA expression that is an evidence for anti-androgenic activity of BPA through AR-antagonist mode of action. However, further studies are required to investigate whether LH involves in regulating anti-androgenic mode of action of BPA. Nelson and Habibi [5] found that ER-beta subtypes are directly regulating VTG production. In addition, ER-beta subtypes can enhance the VTG production through ER-alpha subtype. The present study suggests that BPA at high concentration induce VTG mRNA expression via ER-beta subtypes, because ER-alpha did not show significant increase. Our results also suggested estrogen mimic action of BPA via CYP19A, which convert androgens to estradiol. In the present study, we observed increase of CYP19A when the male goldfish was exposed to 20 ug/L BPA.

Conclusion:

Modes of action of BPA depend on concentration and exposure period. Anti-androgenic mode of action appears at low concentrations through AR-antagonist, but estrogenic activity appears at high concentrations through ER-agonism.

Acknowledgements:

This study was financially supported by GACR 523/09/1793, CENAKVA CZ.1.05/2.1.00/01.0024, 033/2010/Z, 046/2010/Z, and ME10015.

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