Effects of Xenoestrogens on Gonadotropin-Releasing Hormone Neurons During Embryonic Development in Medaka (*Oryzias latipes*)

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Xenoestrogens are chemicals in the environment that can exert estrogenic activity and disrupt the reproductive function of humans and wildlife. In this study, we investigated whether xenoestrogens had any effect on gonadotropoin-releasing hormone (GnRH) gene expression during embryonic development in medaka (*Oryzias latipes*). The medaka fish express three forms of GnRH: GnRH1 regulates reproduction via gonadotropin release, while GnRH2 and GnRH3 are involved in sexual behavior. Here we used a transgenic medaka to track the development of the GnRH3 neurons, in which the green fluorescent protein (GFP) was placed under the control of the *gnrh3* promoter. As the medaka embryos are transparent, the GnRH neurons expressing GFP can be monitored *in vivo* during embryonic development. We treated the *gnrh3-GFP* medaka embryos with various concentrations of bisphenol-A (BPA), nonylphenol (NP), and β-estrogen (E2), and recorded the fluorescence intensity, heart rate, eye development, and the time for the embryos to hatch. We also investigated the mRNA levels of GnRH1, GnRH2, GnRH3, estrogen receptor-alpha (ERα), estrogen receptor-beta (ERβ1), GnRH receptor-I (GnRH-RI), and GnRH receptor-II (GnRH-RII) in above hatchlings with quantitative real-time reverse transcription-PCR (qRT-PCR).

Our results showed that all three xenoestrogens significantly reduced the GFP fluorescence intensity, decreased the heart rate, affected the eye development, and lengthened the time to hatch ($P<0.05$). In addition, the levels of GnRH1, GnRH2, and GnRH3 mRNA were all significantly decreased ($P<0.01$), and the ERα mRNA levels were upregulated ($P<0.05$). No difference was observed in ERβ1, GnRH-RI, and GnRH-RII gene expression. In conclusion, BPA, NP, and E2 can alter the expression of all three GnRH genes, and disrupt the embryonic development.