The Effects of Thyroid Disruption on Reproductive Function in Fathead Minnows

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Introduction

• The endocrine system is made up of the hormone signaling pathways in an organism. Compounds that disrupt normal hormone function are called endocrine disrupting chemicals (EDCs).

• It has been shown that alterations in thyroid signaling can lead to alterations in reproductive function. However, research into thyroid disruption usually does not take this into account (Fig. 1; Duarte-Guterman et al. 2014. Gen Camp Endoc 203:69-85).

• Results of thyroid-reproduction interaction differ between species and are not known for the fathead minnow (Pimephales promelas). This is an issue as this species is commonly used in testing for endocrine disruption.

The objectives of this study were to:
1) assess the effects of chemically induced thyroid disruption on the thyroid signaling pathway
2) determine the reproductive outcomes of thyroid disruption in the fathead minnow

Objective 1 Results: Thyroid disruption

• There were significant changes in expression of deiodinase 2 in brains of PTU exposed females (Fig. 3) and livers of T4 exposed males (Fig. 5).

• These changes in gene expression suggest that the thyroids of these fish were successfully disrupted.

Objective 2 Results: Reproductive function

• There were no significant differences in fecundity, fertilization success, or secondary sexual characteristics. There were significant differences in 48-hour embryo survival (Fig. 6A and 6B). There were also significant differences in GSI of T4 exposed females (Fig. 7).

• There were significant differences in expression of estrogen receptor α (Fig. 8) in liver and 3β-hydroxysteroid dehydrogenase in gonad of males exposed to T4 (Fig. 9). There were also significant differences in aromatase expression in gonads of females exposed to PTU (Fig. 10).

Conclusions

• As a whole, the results show that thyroid disruption in adult fathead minnows can lead to reproductive alterations.

• Reproductive endpoints should be considered along with those traditionally associated with the thyroid in future research on thyroid disrupting EDCs.

• Taking HPT-HPG crosstalk into account may help identify mechanisms of EDCs that cause reproductive alterations but that do not appear to directly impact the HPG axis.

Acknowledgements: TCU Adkins Fellowship and the other members of the Jeffries lab.

Methods

• Adult fathead minnows were exposed to thyroxine (T4) or propylthiouracil (PTU) to promote and inhibit thyroid function, respectively. Doses were Low PTU (2500 µg PTU/g food), High PTU (5000 µg PTU/g food), Low T4 (50 µg T4/g food), and High T4 (100 µg T4/g food).

• Fish were fed dosed food during a 21-day breeding assay. Fecundity (# eggs per female per day), fertilization success, and 48-hour egg survival were monitored.

• At the end of the study, secondary sexual characteristics (Fig. 2) were measured, tissues were collected for gene expression analysis (Table 1) and gonadosomatic index (GSI) was calculated.

Figure 1: Research on the effects of thyroid disruption have traditionally only measured endpoints associated with thyroid function. However, thyroid disruption may also have reproductive effects.

Figure 2: Red arrows indicate secondary sexual characteristics (tubercles and fatpads) of male fathead minnows.

Figure 3: Relative expression of deiodinase 2 mRNA in the brain of T4 exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

Figure 4: Relative expression of deiodinase 2 mRNA in the liver of T4 exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

Figure 5: Relative expression of brain transcription element-binding protein mRNA in the brain of T4 exposed males after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

Figure 6: 48-hour survival of eggs collected from T4 (A) and PTU (B) breeding pairs during the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

Figure 7: Gonadosomatic index of T4 exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

Figure 8: Relative expression of estrogen receptor α mRNA in the liver of T4 exposed males after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.

Figure 9: Relative expression of 3β-hydroxysteroid dehydrogenase mRNA in the gonad of T4 exposed males after the 21-day breeding assay. Error bars represent one standard error. Although a significant difference was detected, a Steel-Dwass post-hoc test was unable to detect specific differences.

Figure 10: Relative expression of aromatase mRNA in the gonad of PTU exposed females after the 21-day breeding assay. Error bars represent one standard error. Groups with the same letter are not significantly different from each other.