Interactions between Thyroid Hormone and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) Signaling during Metamorphosis of *Xenopus laevis*

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ABSTRACT

Dioxin-like compounds are environmental contaminants that elicit toxic effects in vertebrates, including developmental defects, endocrine disruption, and death. Toxicity results from binding to the aryl hydrocarbon receptor (AHR) and subsequent alterations in gene expression. Several species exposed to high levels of TCDD exhibit perturbed thyroid hormone (TH) function. However, the molecular mechanism of dioxin-induced TH disruption is poorly understood, especially during development. Tadpole metamorphosis is a developmental process driven by TH. We used tadpoles of the African clawed frog (*Xenopus laevis*) and an *X. laevis* cell line (XLK-WG) as models to examine effects of dioxin exposure on TH function at the molecular, cellular, and organismal levels. Our results suggest the possibility of a functional interaction between the thyroid hormone receptor (TR) and AHR signaling pathways. Expression of *CYP1A6*, a well-characterized dioxin target gene, was induced at least 300 fold by 100 nM TCDD, and the primary TH target gene, *KLF9,* was induced 5-10 fold by 50 nM TH. *KLF9* mRNA was also induced 2 fold by dioxin. Upon co-exposure to TH and TCDD, *CYP1A6* was induced at least 500 fold, while *KLF9* was induced 12-20 fold. Increased target gene induction following co-exposure of XLK-WG cells to TH and TCDD occurred in the absence of serum in culture media. Thus, this phenomenon was not due to altered interactions with serum-binding proteins and the resulting changes in bioavailability of these compounds. Additionally, target gene induction was sensitive to TR- or AHR-specific antagonists (1-850 and SR1, respectively), demonstrating that molecular effects of dioxin and TH co-exposure depend on TR and AHR agonism. Finally, we examined morphological changes during *X. laevis* metamorphosis to probe the *in vivo* effect of dioxin/TH co-exposure. As our molecular findings predict, dioxin accelerates regression of cultured tail explants.

BACKGROUND

- Amphibian metamorphosis (the transition from tadpole to froglet) is an asynchronous, tissue-specific process driven by thyroid hormone (TH) and mediated by the thyroid receptor.[3]
- Triiodothyronine (T3) is the most biologically active form of thyroid hormone.
- The best characterized TH-responsive gene is Kruppel-Like Factor 9 (KLF-9).[1,4]

- TCDD disrupts TH signaling in humans and mammalian models.[5]
- TCDD elicits toxic effects by binding to the aryl hydrocarbon receptor (AHR).
- Cytochrome P450 1A6 (CYP1A6) is an enzyme involved in the metabolism of xenobiotics, and it is one of the best-characterized AHR target genes.

- Culturing tadpole tail explants provides a way to pair morphological endpoints with our molecular data while separating experimental treatments from endogenously-produced TH.[2]

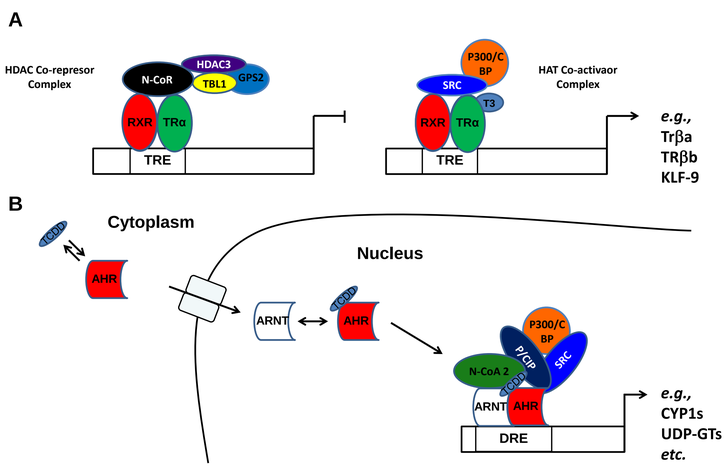
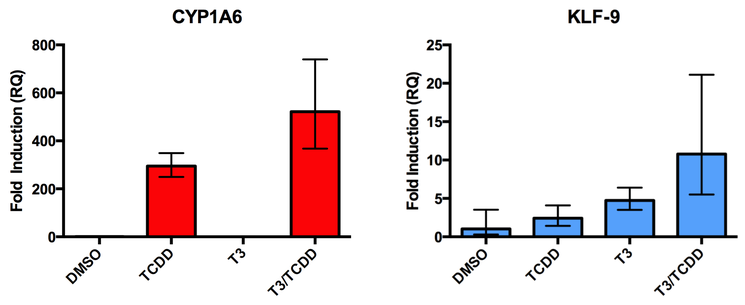


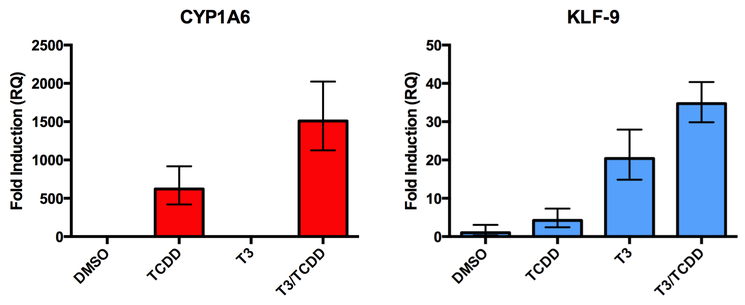
Figure 1. AHR and TR regulate expression of downstream targets. A The TRα dual-function model[3]. TRα/RXR heterodimer is constitutively bound to DNA at thyroid hormone responsive element (TRE). With a host of co-factors, it serves as a transcriptional repressor of genes involved in metamorphosis. In the presence of T3, TRα sheds repressive cofactors and recruits activating cofactors. **B** Unbound AHR resides in the cytosol, but translocates to the nucleus after agonist binding. There it forms a heterodimer with ARNT, binds dioxin responsive elements (DREs) and recruits activating co-factors.

T3/TCDD CO-EXPOSURE

A: Co-exposure to T3/TCDD enhances expression of target genes.

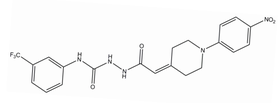


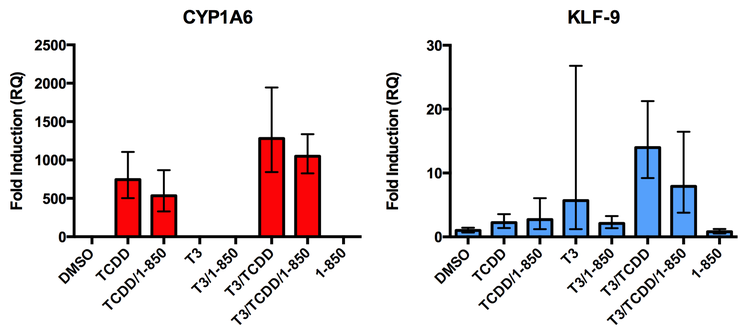
B: Effects of T3/TCDD on target gene expression are independent of serum.



C: Effects of T3/TCDD on target gene expression depend on TR agonism .

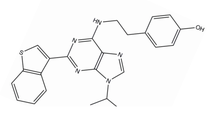
TR Antagonist 1-850:





D: Effects of T3/TCDD on target gene expression depend on AHR agonism.

AHR Antagonist SR1:



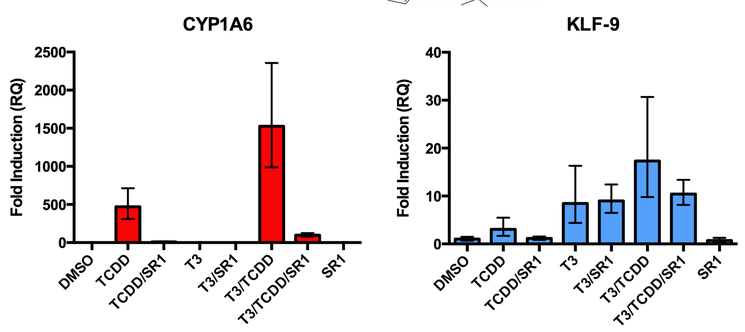


Figure 2. Expression of CYP1A6 and KLF-9 is enhanced with co-treatment of T3 and TCDD. XLK-WG cells were incubated in **(A)** 0.23% DMSO, 100 nM TCDD, 50 nM T3 or 100 nM TCDD and 50 nM T3 in RPMI-1640 with 20% FBS**,** or **(B)** without FBS, **(C)** 1.1% DMSO, 100 nM TCDD, 50 nM T3 and 100 μM 1-850 in RPMI-1640 without FBS, or **(D)** 0.23% DMSO, 100 nM TCDD, 50 nM T3 and 1 μM SR1 in RPMI-1640 without FBS (co-treatments indicated in figure). Cells were treated for 24 hours at 29 °C .Total RNA was purified from XLK-WG cells, treated with DNase, reverse transcribed, and amplified in triplicate by qPCR. Each graph reflects a single biological replicate (n = 3). Error bars represent range of possible RQ values defined by the standard error of the ΔCT.

TAIL EXPLANTS

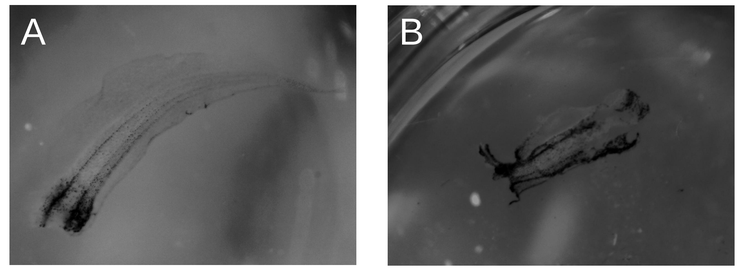


Figure 3. Representative image of a T3/TCDD-treated tadpole tail explant (A) immediately following dissection and (B) seven days post-dissection.

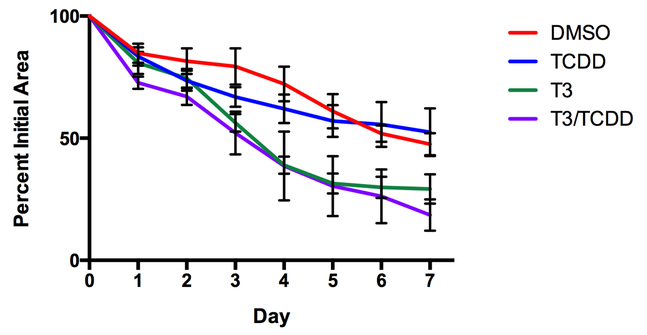


Figure 4. Aaccelerated regression of tadpole tail explants. Tails were harvested from NF stage 52-54 *X. laevis* tadpoles and cultured for 1 week. Tails were treated with DMSO (0.0016%), 100 nM TCDD, 10 nM T3, or 100 nM TCDD and 10 nM T3. Amphibian-strength DMEM containing each treatment was replenished every 12 hours. Tails were imaged daily and area was calculated using ImageJ; this area was then used to calculate percent initial tail area over the 7 day period.

CONCLUSIONS

- Co-treatment with T3 and TCDD enhanced induction of TH- and TCDD-responsive genes above that of either compound alone.
- This increased expression of TR and AHR target genes was not due to changes in bioavailability of either T3 or TCDD as it occurred even in the absence of serum.
- Antagonizing either receptor inhibited the increased induction caused by co-treatment to T3 and TCDD. Thus, enhanced expression of TH- and TCDD-responsive genes is dependent upon agonism of both thyroid hormone receptor and aryl hydrocarbon receptor.
- As molecular findings suggest, tadpole tail regression may be modestly accelerated by TCDD, suggesting that transcriptional changes have morphological impacts during metamorphosis.

FUTURE DIRECTIONS

- Continue tadpole tail explant cultures to confirm preliminary findings
- Studies in whole *X. laevis* tadpoles to pair molecular studies with well-known morphological endpoints during metamorphosis.
- Promoter analysis using luciferase assays to determine AHR binding site on KLF9 promoter of *X. tropicalis*.

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