AHR1-ARNT1 dimerization pair is a major regulator of the response to natural ligands, but not to TCDD in the chicken

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Aim and procedure

Aryl hydrocarbon receptor (AHR) is a ligand dependent transcription factor belonging to the basic helix-loop-helix Per-Arnt-Sim (bHLH/PAS) superfamily. The AHR signaling pathway is initiated as binding of ligands to AHR, forms a heterodimer with the aryl hydrocarbon receptor nuclear translocator (ARNT), and transactivates various genes including cytochrome P450 1A (CYP1A), one of xenobiotics metabolizing enzymes.

This study aims to understand which heterodimerization pair of three AHR isoforms (ckAHR1, ckAHR1β, and ckAHR2) and two ARNT isoforms (*ck*ARNT1 and *ck*ARNT2) responds to natural and endogenous ligands in the chicken, a typical model species of birds. To achieve this, we initially estimated the possibility of ckAHR-ckARNT heterodimerization pairs in several tissues of chickens from the mRNA tissue distribution profiles of each isoform of ckAHRs and ckARNTs. We then measured the transactivation potency of ckAHR-ckARNT heterodimerization pairs in *in vitro* reporter gene assays that each pair of ckAHR and ckARNT isoforms was expressed. We further analyzed the binding mode of several ligands to ckAHR ligand binding domain (LBD) *in silico* to account for the transactivation potency of ckAHR-ckARNT heterodimerization pairs.

Results

In this study, we initially sought to clarify the major chicken AHR-ARNT (ckAHR- ckARNT) pairs by estimating the mRNA tissue distributions of various ckAHR and ckARNT isoforms. Our results indicated that the ckAHR1-ckARNT1 represented the major dimerization pair in most tissues except the brain.

We then measured the transactivation potencies of various ckAHR-ckARNT pairs by natural ligands and 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD), in in vitro reporter gene assays using COS-7 and LMH cell lines. Our results from the in vitro assays demonstrated that the ckAHR1-

ckARNT1 pair was strongly activated by the five natural ligands, namely, 6-formylindolo [3,2-b]carbazole, L-kynurenin, kynurenic acid, indoxyl-3-sulfate, and 1,3,7- tribromodibenzo-p-dioxin, but not by TCDD.

In in silico ligand docking simulations with ckAHR1 homology models, all the natural ligands showed an interaction pattern that was distinct from that observed with anthropogenic DLCs, including TCDD. The interactions between ckAHR1 and DLCs included a greater number of amino acids in the ligand binding domain (LBD), which were shown to not participate in the interactions with the natural ligands. Therefore, these DLC-specific interactions may contribute to differences in the response of the ckAHR1-ckARNT1 pair between the DLCs and natural ligands. Of particular interest is that 1,3,7-TriBDD, a natural dioxin, showed an interaction profile similar to that of other natural ligands. In conclusion, our findings indicate that the ckAHR1-ckARNT1 may be the most important dimerization pair in most tissues for regulating the physiological functions driven by natural ligands, although it was less reactive to TCDD.

Publication/conference presentation

Dong-Hee Koh, Ji-Hee Hwang, Jae-Gon Park, Woo-Seon Song, Hisato Iwata, Eun-Young Kim, AHR1-ARNT1 dimerization pair is a major regulator of the response to natural ligands, but not to TCDD in the chicken, Ecotoxicology and Environmental Safety 201 (2020) 110835.
https://doi.org/10.1016/j.ecoenv.2020.110835

Perspectives in future

To our knowledge, this is the first study that investigated the avian AHR isoform-specific transactivation potencies of natural ligands. In this study, we estimated which ckAHR-ckARNT heterodimers were involved in the response to ligands in each tissue based on the mRNA expression levels. Our results suggested that the ckAHR1-ckARNT1 pair, with high mRNA expression and ligand sensitivity, is a major regulator of the physiological responses to natural ligands in the chicken. Unlike the AHRs present in other species, the ckAHR1- ckARNT1 pair was shown to have a weak response to TCDD, compared with natural ligands, in the chicken. This is probably due to ckAHR isoform-specific interactions with anthropogenic DLCs and ckAHR1- specific amino acid sequences. On the other hand, the ckAHR1- ckARNT2 pair is likely to act as an important regulator of TCDD and natural ligands in the brain and tissues where ckARNT2 is expressed to some extent. Further understanding of the ligand preferences of the AHR-ARNT heterodimerization pairs may contribute to disease treatment and drug development targeting the AHR signaling pathway in avian species.