Impact of 3,3'-Diindolylmethane on AHR activation: Insights into natural ligand responses and hepatic transcriptome changes in chicken embryos

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Purposes

The research primarily aimed to understand how natural ligands interact with the AHR in chicken embryos. This is crucial because the AHR can mediate both physiological responses and toxic effects. By investigating the response of chicken AHR to various natural ligands, the study sought to address a knowledge gap in our understanding of AHR's diverse roles in avian species. This is particularly relevant because domestic chicken AHR is known to be more sensitive to dioxins than that of wild birds.

In this study, we aimed to:

Characterize the response of chicken AHR to a range of natural ligands: This involved measuring the ability of these ligands to activate AHR, providing insights into their potential to influence AHR-mediated processes in chicken embryos.

Investigate the effects of 3,3'-Diindolylmethane (DIM) on chicken embryos: DIM was chosen as a focus due to its strong AHR activation potential. The study determined the phenotypic effects of DIM exposure and analyze the changes in the hepatic transcriptome, which provides a snapshot of gene expression activity.

Enhance understanding of AHR's role: By studying the interaction of natural ligands with AHR, the research contributes to a broader understanding of how environmental factors can influence AHR activity and potentially impact ecological health.

Methods

The study employed both of in vitro and in vivo approaches to investigate AHR activation and its effects in chicken embryos. The methods used include:

In vitro reporter gene assays: These assays were conducted to evaluate the transactivation potencies of seven different natural ligands on chicken AHR. The luciferase activity in response to the different ligands was measured. This allowed to quantify the ability of each ligand to activate chicken AHR.

Chicken embryo exposure and phenotypic analysis: Chicken embryos were exposed to DIM, and their development was monitored for any observable phenotypic changes.

Hepatic transcriptome analysis: RNA sequencing was used to analyze gene expression changes in the livers of chicken embryos exposed to DIM. This allowed to identify differentially expressed genes (DEGs).

Pathway and network analyses: Bioinformatics tools were used to analyze the DEGs and identify the biological pathways and networks affected by DIM exposure. Through these analyses, we evaluated the functional consequences of AHR activation by DIM in the liver.

Results

The in vitro reporter gene assays revealed that the natural ligands tested exhibited varying abilities to activate chicken AHR. This suggests that different natural compounds can have distinct effects on AHR signaling. DIM emerged as the most potent activator of chicken AHR among the tested ligands. This finding highlighted DIM's potential to significantly influence AHR-mediated processes in chicken embryos. Exposure to DIM resulted in significant changes in the hepatic transcriptome of chicken embryos. We successfully identified 936 of DEGs, indicating that DIM has a broad impact on liver function. Pathway and network analyses of the DEGs suggested that DIM exposure activates various processes in the liver, including the regulation of cell proliferation, angiogenesis, metabolic responses, and immune system. This highlights the pleiotropic effects of AHR activation by DIM.

Future Challenges

This research provides valuable insights into the interaction of natural ligands with AHR in chicken embryos. Further research is needed to investigate the combined effects of natural ligands and environmental pollutants on AHR activation and the potential for synergistic or antagonistic interactions. Understanding the mechanisms of AHR activation can help in developing strategies to protect avian populations from the harmful effects of environmental contaminants.