BIOLOGICAL AND TOXICOLOGICAL CONSEQUENCES OF Ah RECEPTOR ACTIVATION: JUST HOW COMPLICATED CAN ONE RECEPTOR GET?

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Abstract

The Ah receptor (AhR) is a ligand-dependent transcription factor receptor that mediates a wide range of biological and toxicological effects produced by a structurally-diverse synthetic and naturally-occurring chemicals. While the overall mechanism of action of the AhR has been extensively studied and involves a classical nuclear receptor mechanism of action (i.e. ligand-dependent nuclear localization, protein dimerization, binding of liganded receptor as a dimer to its specific DNA recognition sequence and activation of gene expression), details of the exact molecular events that result in most AhR-dependent biochemical, physiological and toxicological effects are generally lacking. While ongoing research efforts continue to describe an ever-expanding list of ligand- and tissue-specific and AhR-dependent diverse biological and toxicological effects and seemingly complicate the story even more. However, these same studies are also identifying and characterizing new pathways and molecular mechanisms by which the AhR exerts its actions and both are providing insights and questions regarding the diversity in responses following ligand-dependent activation of the AhR signal transduction system.

Overview

The aryl hydrocarbon receptor (AhR) is a soluble, intracellular, ligand-dependent transcription factor that mediates a diverse array of biological and toxicological effects in a wide variety of species and tissues, including (but not limited to) tumor promotion, teratogenicity, immuno- and dermal toxicity, wasting, lethality, modulation of cell growth, proliferation and differentiation, alterations in endocrine homeostasis, reduction in steroid hormone-dependent responses and induction and repression expression of numerous genes. While research over the past three decades has dramatically increased our understanding of the basic mechanism by which the AhR produces some of these effects, the focus of most studies has been primarily on its ability to induce gene expression. Mechanistically, the unliganded AhR exists in the cytosol as an inactive multiprotein complex consisting of the AhR and several other proteins (Hsp90, XAP-2 and p23). Ligand binding stimulates nuclear translocation of the AhR protein complex and dimerization of the AhR with the nuclear protein Arnt (which stimulates release of the AhR from its protein complex). Binding of the resulting ligand:AhR:ARNT complex to its specific DNA recognition sequence, the dioxin response element (DRE), leading to recruitment of nuclear protein factors (coactivators) and increased transcriptional activation of downstream genes.[1] While the best studied AhR responsive genes are enzymes involved in drug and chemical metabolism (i.e., cytochrome P4501A1 (CYP1A1) and others), gene array studies have identified a large number of ligand-inducible, AhR-responsive gene products. Studies with knockout mice convincingly demonstrate the absolute requirement of the AhR, its nuclear translocation and/or DNA binding in the toxic and biological effects of AhR agonists[2], at first glance it would appear surprising that in the 33 years since the AhR was first identified and characterized by Poland and coworkers[3], that the molecular mechanisms by which the AhR produces its characteristic spectrum of toxic and biological effects still remains to be elucidated. However, given that these ligand- and AhR-dependent effects do not appear to be directly caused by the primary gene products induced by the AhR signal transduction pathway, the speices- and tissue-specific differences in many responses, the significant delay in the manifestation of many of the adverse effects of some AhR ligands (i.e. halogenated aromatic hydrocarbons (HAHs), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)), coupled with relatively recent results demonstrating that the ligand-activated AhR can induce and inhibit expression of a large number of genes[1] and the observations that the AhR can affect and/or be affected by diverse intracellular signaling and biochemical pathways[4-9], it would actually be more surprising had all of the exact mechanisms responsible for AhR-dependent biochemical and

toxic effects been resolved.

While ligand-dependent induction of AhR-dependent gene expression by the "classical" AhR:Arnt:DRE mechanism can produce a variety of biological and toxicological effects, many of these effects are secondary, in that the gene products induced by the AhR are responsible for mediating the observed effects. Induction of CYP1A1 and other AhR-responsive metabolic enzymes can indirectly result in changes in the functionality cellular signaling and receptor pathways. For example, while the antiestrogenic action of TCDD and other AhR ligands is multifactorial in nature, AhR-dependent increases in CYP1A1/CYP1B1 have been shown to result in increased catabolism of estrogen and thus an overall reduction in estrogen response[1,2,10]. Ligand-dependent induction of expression of CYP1A1 and the resulting increase in CYP1A1 enzymatic activity has been observed to result in release of reactive oxygen species (ROS) which is suggested to account for the oxidative stress observed following exposure to AhR agonists. Not only has the increase in CYP1A1-dependent ROS levels been shown to result in an increase in oxidative DNA damage[11], but increased ROS can lead to activation of intracellular signaling kinase pathways (JNK and NF-kB for example) and thus indirectly changes in gene expression and cellular responses[4,5,9]. In fact, the recent demonstration that activation of AhR-dependent gene expression can lead to increased levels of CYP1A1 in the inner mitochrondrial membrane (in addition to the endoplasmic reticulum) can lead to mitochrondrial dysfunction (alternations in transmembrane potential, alterations in energy production and stress signaling). While these TCDD-dependent effects are suggested to contribute to tumor progression in vivo[12], one can envision how alterations in mitochondrial energy production could contribute to the "wasting syndrome" associated with exposure to AhR ligands. The results of Uno and coworkers suggest a role for CYP1A1 in wasting (CYP1A1 knockout mice showed no wasting with TCDD exposure)[13], this mitochondrial CYP1A1-ROS wasting hypothesis remains to be confirmed.

While the "classical" AhR-Arnt-DRE signaling pathway plays a major regulatory role in the majority of AhR-dependent responses, recent studies have demonstrated the ability of the liganded AhR and/or AhR:Arnt complex to differentially affect gene expression in a "non-classical" manner within the cell nucleus. For example, one mechanism by which the AhR produces antiestrogenic effects is through binding of liganded AhR:Arnt complex to DNA sequences that have been referred to as "inhibitory DREs (iDREs)"[1,10,14]. Ligand:AhR:Arnt:iDRE complex formation is not only non-productive (i.e., it does not stimulate expression of the adjacent gene), but it directly interferes with the ability of other transcription factors (i.e., estrogen receptors) to bind to its DNA recognition site immediately adjacent to these iDREs, thus repressing estrogen-dependent induction of expression of this gene. The ability of the AhR or AhR:Arnt complex to stimulate or suppress the ability of other transcription factors to activate gene expression by acting sequestering key coactivators or DNA binding partner proteins (as has been reported for the hypoxia inducible factor HIF-1, estrogen receptor (ER), NF-kB, E2F) or to interact with other nuclear factors needed for their functional activity and or to directly bind to a DNA bound transcription factor and alter its functionality (i.e ERs) have also been reported[1,2,5,9]. Modulation of the functionality of these specific transcription factors will significantly impact cell proliferation, differentiation, cell cycle, stress and hormone responses and responsiveness, and differences in these systems between cell types would contribute to the diversity of cell-specific responses to AhR ligands.

The ability of TCDD and other AhR ligands (i.e. polycyclic aromatic hydrocarbson (PAHs)) produce both rapid (within minutes) and sustained calcium influx into exposed cells and the observation that TCDD can produce effects in AhR knockout mice indicates that these chemicals can also produce significant effects in an AhR-independent manner[5]. Calcium-dependent stimulation of cAMP production from adenylate cyclase, and the subsequent activation of protein kinase A (PKA) can produce a diverse spectrum of intracellular responses that could contribute to the overall biochemical and toxic effects of AhR ligand exposure. In fact, recent studies have reported a role for PKA in the inflammatory response to TCDD and other AhR agonist and involvement of the AhR in stimulating the cAMP and PKA pathway[7,15]. The ability of PKA to stimulate activity of the epidermal growth factor receptor (EGFR) and its downstream events, coupled with the ability of PKA and the AhR to stimulate expression of known EGFR ligands would contribute to the activation of the EGFR-dependent MAP kinase pathway and MAPK-dependent changes in gene expression[4,5]. In fact, expression of cycloxygenase 2 (Cox-2), a gene product known to promote cell survival and to be involved in skin carcinogenesis, can be directly induced by the classical AhR signaling pathway (along with CYP1A1, known to be involved in

carcinogenesis), but also indirectly through the EGFR-mediated pathway. Together, these AhR-responsive pathways can contribute to the carcinogenesis of AhR ligands. Interestingly, since cAMP has also been recently shown to activate the AhR independently of exogenous ligand[2], increased cAMP could also increase the overall AhR-dependent response and if the cAMP-activated AhR can differentially activate gene expression (compared to ligand-activated AhR), this would add another level of complexity to the observed diversity in AhR responses. Interestingly, while recent evidence has indicated that TCDD can increase intracellular calcium levels through its ability to open plasma membrane calcium channels and intracellular calcium (RyR) channels stores[6,12], the exact mechanism by which TCDD and perhaps other AhR ligands can do this remains an open question. However, studies using cells from AhR knockout animals should provide the optimal model to examine this mechanism.

While the above mechanisms represent some of the pathways that are or can be affected by AhR ligands and can contribute to the observed diversity in AhR response, not all AhR ligands produce the same spectrum of biochemical and toxicological responses. HAHs, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) and related polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls, represent the best characterized and highest affinity ligands for the AhR and these HAHs produce the full spectrum of AhR-dependent toxic and biological effects. In contrast, PAHs and PAH-like chemicals, such as 3-methylcholanthrene, β-napthoflavone, indolo[3,2-b]carbazole and others are also relatively high affinity AhR ligands, and while they produce a similar spectrum of biological effects as the HAHs (i.e. alterations in gene expression and others) albeit more transiently, these chemicals rarely produce the AhR-dependent toxic effects observed with HAHs (i.e. wasting, teratogenicity, dermal toxicity, lethality, thymic involution). One major difference between these chemicals is that HAHs are poorly metabolized, while PAHs and other structurally diverse AhR ligands are readily degraded by metabolism. Gene induction experiments demonstrate that TCDD and related HAHs induce persistent activation of AhR-dependent gene expression, while that of PAHs, PAH-like chemicals and other structurally diverse ligands induce AhR-dependent gene expression only transiently. These results, combined with the observation that metabolically labile AhR ligands can produce similar (albeit transient) effects on the majority of the alternative AhR signaling pathways and AhR-associated pathways described above, strongly suggests that AhR-dependent toxicity of HAHs is predominantly driven by their metabolic persistence leading to persistent activation of AhR present in the responsive cells[8].

While the metabolic persistence of AhR agonists appears to be the major driver for the adverse effects of HAHs compared to PAHs, other aspects should still be considered when assessing the differential responsiveness of HAHs and PAHs and their ability to produce biological and toxicological responses. Recent work in our laboratory is consistent with the hypothesis that HAHs and non-HAH ligands for the AhR bind distinctly differently within the ligand binding pocket of the AhR. This differential binding could lead to ligand-dependent differences in the overall structure of the AhR that may contribute to differences in its overall functionality (i.e. differences in coactivator recruitment and transcriptional activity), similar to that observed for some steroid hormone receptors. However this remains to be confirmed. It is also naïve to believe that a single chemical will produce effects in a cell/tissue or animal by a single mechanism (while this remains to be demonstrated for other AhR ligands, TCDD and some PAHs clearly produce effects by at least two mechanisms), and as such, one can envision that HAHs could produce a unique HAH-specific cellular response(s) that in combination with activation of the AhR leads to the spectrum of AhR-dependent toxicity. The inability of nonHAH AhR ligands to produce the HAH-specific response would still result in these ligands inducing a spectrum of AhR-dependent biological responses (similar to that observed with HAHs), but not the toxic effects. While this has not yet been examined, the lack of TCDD-dependent toxicity in AhR knockout animals would not be inconsistent with this hypothesis as AhR activation would be a necessary component to the toxic mechanism of action, irrespective of the ligand.

Overall, the continued analysis of the AhR and AhR signal transduction pathway have provided an increased understanding of basic molecular mechanisms of how structurally diverse AhR ligands can bind to and activate the AhR and produce species-, tissue- and ligand-specific toxic and biological effects. These studies will also continue to increase our knowledge of the endogenous physiological role(s) and ligands for the AhR and AhR signaling pathway.

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