Endogenous Functions of the Aryl Hydrocarbon Receiver (AHR): Intersection of Cytochrome P450 1 (CYP1)-metabolized Eicosanoids and AHR Biology*5

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The AHR,2 a ligand-activated transcription factor, was first identified indirectly in the 1970s; the mouse and human genes were cloned in the early 1990s. Molecular mechanisms and biological consequences of AHR-mediated regulation of mammalian cytochrome P450 enzymes by foreign chemicals (e.g. polycyclic aromatic hydrocarbons and dioxins) have been studied extensively. Binding of such ligands to AHR leads to transcriptional activation of CYP1A1, CYP1A2, and CYP1B1; in turn, these enzymes catalyze oxidative detoxication or activation of most ligands. From the start, AHR endogenous ligands and functions were postulated; although this was initially controversial, the robust phenotype of Ahr−/− knock-out mice provided clear evidence of physiological roles (and endogenous ligands) for AHR. AHR has numerous important endogenous functions: during conception and embryonic and fetal development; in the immune, cardiovascular, neural, and reproductive systems; and in hepatocytes, skin cells, and adipocytes. These myriad AHR-mediated processes mirror the vast universe of action of the eicosanoids, lipid mediators known to undergo cytochrome P450-dependent oxidation. We propose that many endogenous and exogenous cellular stimuli lead to (i) AHR-dependent CYP1-independent eicosanoid synthesis and degradation; and (ii) AHR-dependent CYP1-independent (eicosanoid-dependent and -independent) responses. These two pathways can be delineated from one another in genetic models: the former is absent in the recently characterized Cyp1a1/1a2/1b1−/− triple-knock-out mouse, and the latter is absent in the Ahr−/− knock-out mouse. Identification of specific eicosanoids whose synthesis or degradation is carried out by each CYP1 enzyme should allow for identification of physiological endogenous AHR ligands. (For “History and Background,” see supplemental material (Box 1).)

Eicosanoids: Underappreciated Mediators of Everything

Oxynated fatty acids are widely employed as signaling molecules by prokaryotes and eukaryotes (1). Among these, the eicosanoids are bioactive oxygenated derivatives of ω-6 or ω-3 essential fatty acids. Although the first identified eicosanoids were derived from fatty acids with 20 carbon atoms (hence, “eicosa,” Greek for “twenty”), such as arachidonic acid, eicosapentaenoic acid, and dihomo-γ-linolenic acid, related derivatives of 22-carbon atom essential fatty acids (e.g. docosahexaenoic acid) carry the same moniker.

Eicosanoids are local hormones released by most vertebrate (and some invertebrate) cells, which act in autocrine or paracrine fashion and then become rapidly inactivated. Potent in the nanomolar range, at least 13 categories of eicosanoids have been classified to date (supplemental Table S2), and if one includes all possible stereoisomers, the total number of eicosanoids now exceeds 150. Supplemental Table S2 lists the incredibly large number of critical life functions mediated via eicosanoids, showing some degree of specificity of function among the 13 categories. In terms of immunity, both initiation and resolution of inflammatory processes are under the critical control of eicosanoids (2).

Generally, eicosanoids are not stored within cells but are synthesized as needed; one exception is that red blood cells are reservoirs for cis- and trans-epoxyeicosatrienoic acids that can quickly be released (3). Stimuli that initiate eicosanoid biosynthesis and release include mechanical trauma, cytokines, growth factors, xenobiotics, and even other eicosanoids. Such stimuli trigger the activation of phospholipases at the cell or nuclear membrane, where fatty acid precursors of eicosanoids are incorporated as esters into larger molecules (phospholipids and diacylglycerol), whereupon the phospholipase catalyzes ester hydrolysis of phospholipid (via phospholipase A2) or diacylglycerol (via diacylglycerol lipase). The rate-limiting step for eicosanoid formation appears to be this hydrolysis, which frees eicosanoid precursors.

Do AHR-dependent CYP1-dependent Responses Reflect Eicosanoid Metabolism?

Eicosanoids exert complex control over virtually all life functions (supplemental Table S2). Interestingly, the list of processes that exhibit abnormalities when AHR is absent or are modulated by activation of AHR (supplemental Table S1) is quite similar to the list of eicosanoid-mediated functions (supplemental Table S2).

Generic Signals and Responses—Any of thousands of different exogenous signals affecting a cell can be viewed as a stimulus that is “perceived” by XTFs; these include the AHR, constitutive androstane receptor, hepatocyte nuclear factor, forkhead box, liver X receptor, peroxisome proliferator-activated receptor, farnesoid X receptor, pregnane X receptor, and related
families (Fig. 1A), which then regulate various downstream targets. The concept of xenobiotic-related transporters and XTFs has been recently reviewed (4). We envision the downstream events to include CYP1-, CYP2-, CYP3-, and CYP4-mediated synthesis and degradation of specific eicosanoids (5) as well as responses that are independent of these enzymes (Fig. 1A).

**AHR Signaling and Responses**—Although AHR binds to AHR response elements in hundreds of genes throughout the genome, the three CYP1 genes conserved in all mammals (CYP1A1, CYP1A2, and CYP1B1) are both among the most highly induced of the panel of AHR-activated genes and central to the oxidative metabolism of many AHR ligands. Hence, we envision that signals received by AHR lead to downstream events (Fig. 1, B and C), which include AHR-dependent CYP1-dependent synthesis and degradation of specific eicosanoids and AHR-dependent CYP1-independent (eicosanoid-dependent and -independent) responses.

**Cyclooxygenases and ALOXs**—Although considerable emphasis has been placed on the role of cyclooxygenase-1 and -2 and the ALOXs in eicosanoid synthesis and degradation (Fig. 1C), it has been experimentally demonstrated that dozens of members of the CYP1, CYP2, CYP3, and CYP4 families also participate in these processes (Table 1) (5). Every member of these four CYP families might be involved in eicosanoid metabolism, perhaps with redundancy (5). Pursuant to the present review about AHR endogenous functions, the precise steps catalyzed by CYP1A1, CYP1A2, and CYP1B1 remain to be demonstrated. In fact, the large number, complexity of pathways, extreme lability, and similarity of the various eicosanoid chemical structures and stereoisomers all contribute to the difficulty of successful research in this arena.

**Cyp1a1/1a2/1b1−/− Triple-knock-out Mouse as a Model System**

How might AHR-dependent CYP1-dependent responses be dissected from AHR-dependent CYP1-independent responses (Fig. 1)? One excellent model system is the recently characterized Cyp1a1/1a2/1b1−/− triple-knock-out mouse (6). The other model system is the Ahr−/− knock-out mouse (reviewed in Refs.
The CYP1 enzymes participate in immune dysregulation and enhanced susceptibility to infection, whereas an AHR-dependent CYP1-independent process is responsible for increased risk of the PDV and AV shunts.

**What Are the “True” Endogenous Ligands for AHR?**

Various classes of endogenous compounds shown to induce CYP1 and/or activate AHR include (a) tryptophan metabolites, other indole-containing molecules, and phenylethylamines (16); (b) tetrapyroles such as bilirubin and biliverdin; (c) sterols such as 7-ketocholesterol and the horse steroid equilenin; (d) fatty acid metabolites, including at least six different prostaglandins (17) and lipoxin A₄; and (e) the ubiquitous second messenger cAMP (reviewed in Refs. 9 and 18). The dose of such putative inducers in the intact animal, the concentrations needed in cell cultures, and the dissociation constant of binding (Kᵦ) for the majority of these candidates are, however, usually not as low as one would expect for physiologically relevant AHR ligands. We believe that there is likely to be cell- and tissue-type specificity and redundancy for endogenous AHR ligands. It should now be possible to identify CYP1-mediated AHR ligands by comparing metabolite profiles from an assortment of tissues of the wild-type mouse with those of various combinations of the Cyp1 knock-out lines that are now available.

**Other Evidence of Endogenous Functions of AHR**—In untreated mice, the highest AHR level in any cell type is seen in the oocyte (19), and incredibly high levels of CYP1A1 mRNA are found in the oocyte after fertilization (20). Interestingly, retinoic acid-induced differentiation in cultured cells and hepatic regeneration in the intact mouse are both associated with up-regulation of CYP1A1 in the absence of an exogenous inducer (21). Physical fluid shear stress in the animal (9, 18) and UVB radiation (290–320 nm) (22), as well as changing of growth medium in cultured cells, also up-regulate CYP1 and/or activate AHR (reviewed in Ref. 9). Needless to say, division of the oocyte to the two-cell stage, further cell divisions, cell differentiation and proliferation, mechanical trauma and fluid shear stress, and UV radiation all involve eicosanoids (supplemental Table S2).

**Eicosanoid Action in the GI Tract**—In studies of Arnt⁻/⁻ conditional knock-out mice in which Cre recombinase is driven by a villin promoter, the result is complete ablation of ARNT in GI epithelial enterocytes; curiously, CYP1A1 mRNA and enzyme activity are markedly elevated in virtually all tissues of this mouse, other than the enterocyte (23). CYP1A1 induction is greater with indole-3-carbinol added to the diet (23). These observations fit well with the theme of AHR, CYP1, and eicosanoid action in the GI tract. Interestingly, prostaglandin E1 (e.g. very high in sheep testicle) yet exists only in trace amounts in humans. In addition to six arachidonate lipoxigenase genes, ALOX5, ALOX12, ALOX12B, ALOX15, ALOX15B, and ALOX16, which are orthologous between the human and mouse genomes, the mouse genome has a seventh gene, Alox12a. EETs, epoxyeicosatrienoic acids; HETEs, hydroxyeicosatetraenoic acids; DHETEs, dihydroxyeicosatrienoic acids.
TABLE 1
Specific catalytic evidence for eicosanoid metabolism by members of the vertebrate CYP1, CYP2, CYP3, and CYP4 families

This search is not intended to be all inclusive but merely to illustrate the breadth of work in this field. The early pioneering work of Michal Laniado-Schwartzman, Volker Ulrich, Jorge Capdevila, Yoshihiko Funae, and Seichū Yoshida is especially notable. The studies cited here were selected mostly on the basis of purified or recombinant cytochrome P450 protein. Even Xenopus oocytes exhibit P450-mediated formation of hydroxyeicosatetraenoic acids (70). Interestingly, CYP-mediated metabolism of eicosapentaenoic acid in Caenorhabditis elegans has also been reported (71); this P450 is most closely related to the CYP4 family (D. R. Nelson, personal communication). ALOX enzymes also exist in prokaryotes (72) and prokaryotes (73). EETs, epoxyscleratetraenoic acids; HETEs, hydroxyeicosatetraenoic acids; HPETEs, hydroperoxyeicosatetraenoic acids.

| CYP subfamily and eicosanoid categories | Ref. |
|----------------------------------------|-----|
| **CYP1A**                              |     |
| Prostaglandins                         | 30, 33 |
| Arachidonic acid, prostaglandins       | 31 |
| EETs, HETEs                            | 32, 35 |
| Arachidonic acid, EETs, eicosapentaenoic acid | 34 |
| Eicosapentaenoic and docosahexaenoic acids | 36 |
| **CYP1B**                              |     |
| EETs, HETEs                            | 37 |
| **CYP2A**                              |     |
| Arachidonic acid, prostaglandins       | 31 |
| **CYP2B**                              |     |
| Arachidonic acid, prostaglandins       | 31 |
| HPETEs                                 | 38 |
| EETs, HETEs                            | 39 |
| EETs                                   | 40, 41 |
| **CYP2C**                              |     |
| Arachidonic acid, prostaglandins       | 31 |
| Arachidonic acid                       | 42 |
| EETs, HETEs                            | 32 |
| Prostaglandins                         | 43 |
| EETs                                   | 44 |
| **CYP2D**                              |     |
| Arachidonic acid, HETEs                | 45, 46 |
| **CYP2E**                              |     |
| Arachidonic acid, prostaglandins       | 31 |
| Arachidonic acid, EETs, HETEs          | 47 |
| EETs, HETEs                            | 32 |
| Prostaglandins                         | 33 |
| **CYP2F**                              |     |
| EETs                                   | 48, 51 |
| Arachidonic acid, HETEs                | 49 |
| Arachidonic acid                       | 50 |
| **CYP2G**                              |     |
| Arachidonic acid, EETs, HETEs          | 52 |
| **CYP2U**                              |     |
| Arachidonic acid                       | 53 |
| **CYP2W**                              |     |
| Arachidonic acid                       | 54 |
| **CYP3A**                              |     |
| Arachidonic acid, prostaglandins       | 31 |
| Prostaglandins                         | 33 |
| **CYP3B**                              |     |
| Prostaglandins                         | 43, 55 |
| Arachidonic acid, prostaglandins       | 56 |
| EETs                                   | 11 |
| HETEs                                  | 57 |
| **CYP3D**                              |     |
| EETs, HETEs                            | 58, 59 |
| **CYP4A**                              |     |
| Leukotrienes, prostaglandins           | 60 |
| Leukotrienes                           | 61 |
| Prostaglandins                         | 43, 62, 63 |
| Arachidonic acid                       | 64 |
| Leukotrienes, lipoxins, HETEs          | 65 |
| EETs                                   | 66 |
| Eicosapentaenoic and docosahexaenoic acids | 67 |
| Lipoxins, HETEs                        | 68 |
| HETEs                                  | 69 |

References
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The process of food absorption and digestion involves continuous low-grade inflammation (and resolution of inflammation) in the GI tract. The allochemical indole-3-carbolin also causes eicosanoid release. Removal of ARNT from the enteroctye blocks AHR function and therefore CYP1 expression in that cell type; we hypothesize that, as a result, eicosanoids normally synthesized or degraded by the enteroocyte CYP1 enzymes are released throughout the rest of the animal and that these eicosanoids are AHR ligands that then cause CYP1A1 up-regulation in virtually every cell type except ARNT-deficient enteroctyes.

**What Happens Downstream of Eicosanoids?**

Beyond the scope of this review, eicosanoid synthesis and degradation are not the end of the story. Specific receptors for prostaglandin (24–26) and for other eicosanoids (27–29) are being characterized, many of which are G-protein-coupled receptors, but the field is still in its infancy; perhaps other heretofore unknown perception systems also exist (Fig. 1C). Our understanding about the release of w-6 and w-3 fatty acids from phospholipids and diacylglycerol and about the synthesis and degradation of specific eicosanoids by certain ALOX and CYP enzymes may well lead to the design of new drug targets. Similarly, a better understanding of eicosanoid receptors and their targets should also result in more knowledge and possible new drug targets in treating various human disorders, including specific types of cancer.

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