Regulation of Dioxin Receptor Function

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Introduction

The dioxin receptor is a ligand-activated transcription factor that belongs to the basic helix-loop-helix/PAS (bHLH/PAS) family of proteins (1, 2). bHLH factors can bind DNA as homo- and/or heterodimers. The latter seems to be true for the dioxin receptor since a structurally related non-dioxin-binding protein denoted Arnt (4), has been shown to be a part of the DNA-binding form of the ligand-activated receptor, and is necessary for DNA recognition (3). A structural similarity has been found between the dioxin receptor, Arnt and the Drosophila proteins Sim and Per (4). The homologous region called PAS (Per, Arnt, Sim) is located in the N-terminus of these four proteins.

The inactive, ligand-free dioxin receptor is a stable heteromeric complex with the 90 kDa heat-shock protein (hsp90; 5). Upon ligand binding the dioxin receptor is activated to its DNA-binding state by sequential release of hsp90 and heteromerization with Arnt. The dioxin receptor/Arnt heteromere specifically binds xenobiotic response elements (XREs) located in, for instance, the promoter region of the CYP1A1 gene (6) encoding the cytochrome P4501A1 protein.

Most studies of P4501A1 activity and inducibility have been performed using hepatic cells since liver is the tissue that has the highest concentration of P4501A1 (reviewed by 7). However, tissue- and cell type-specific differences with regard to P4501A1 inducibility by polychlorinated hydrocarbons have become evident. For example, P4501A1 inducibility is dependent upon the differentiated state of keratinocytes while other cell types, i.e. normal fibroblasts are non-responsive to dioxin (8, 9).

Results and Discussion

Normal fibroblasts do not respond to dioxin with increased cytochrome P4501A1 expression although both the dioxin receptor and its partner factor Arnt are expressed in these cells, as demonstrated by both RNA blot analysis and DNA binding assays. Since the expression levels of these two proteins in fibroblasts are comparable to those present in, for instance, inducible keratinocytes or HepG2 cells, the basis for non-responsiveness of fibroblasts is not related to the relative abundance of dioxin receptor or Arnt.

Although the endogenous dioxin receptor can be activated to its DNA-binding form upon exposure to the dioxin receptor ligand TCDF this response does not lead to induction of transcription of the CYP1A1 target gene or an increased expression of XRE containing minimal promoter constructs in fibroblasts. Thus, it was important to establish that functional properties of the dioxin receptor, other than its DNA binding activity, were not inhibited by post-translational mechanisms. By fusing fragments of the dioxin receptor and Arnt that lack their corresponding bHLH motifs with a heterologous DNA-binding domain the independent function of these two factors can be studied in transfected cells. Importantly, these experiments show that the chimeric dioxin receptor is conditionally regulated by dioxin and that the constitutive transcriptional activation function of Arnt is similar in fibroblasts in comparison to responsive HepG2 cells. In addition, Arnt is functional as a partner factor to HIF-1a since $CoCl_2$ was able to induce erythropoietin and aldolase A mRNA levels.

In addition to the dioxin receptor, two novel constitutive protein-XRE complexes was detected. The fibroblast XRE-binding factor(s) were immunochemically distinctive from the dioxin receptor but exhibited indistinguishable DNA binding specificity. These data are compatible with a model where the P4501A1 is noninducible in fibroblasts due to the presence of a putative repressor(s) that may compete effectively with the dioxin receptor for binding to the XRE.

References

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