binds neither [^{3}H]TCDD nor [^{3}H]B-naphthoflavone (BNF). However, preliminary studies indicate the *in vitro*-expressed clam AHR does exhibit sequence-specific interactions with a mammalian xenobiotic response element (XRE) in the absence of exogenous ligand. *in vitro*-expressed *D. melanogaster* and *C. elegans* AHR homologues also lack specific binding to [^{3}H]TCDD and [^{3}H]BNF. The lack of specific binding to these prototypical AHR ligands appears to be a property shared by known invertebrate AHR homologues, distinguishing them from vertebrate AHRs. Comparative studies of phylogenetically diverse organisms may help identify an endogenous ligand(s) and the physiological role(s) for this protein.

1260 HORMONAL ACTIVATION OF LACTATE DEHYDROGENASE GENE EXPRESSION IN BREAST CANCER CELLS.

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In previous studies, we identified the -92 to -37 region of the lactate dehydrogenase (LDH) gene promoter as estrogen (E2)-responsive in transient transfection studies in MCF-7 human breast cancer cells. This promoter sequence contains a cAMP response element (CRE) and nuclear extracts from MCF-7 cells bound [^{32}P]-58/-36 (CRE-sequence from LDH promoter) in gel mobility shift assays to form a broad retarded band which was decreased in intensity after competition with an unlabeled consensus CRE oligonucleotide. The retarded band was supershifted after incubation of nuclear extracts with antibodies that bound ATF-1 and CREB. Constitutively active protein kinase A and an inducer of cAMP (cholera toxin) induced reporter gene activity in breast cancer cells transfected with the E2-responsive pLDH1 construct (contains the -58 to +9 LDH gene promoter insert) confirming that cAMP-PKA induction also activates the CRE. Hormonal activation of pLDH1 and related constructs was also inhibited by cotransfection with KREB, a dominant negative form of CREB. Activation of CREB and ATF-1 by E2 through non-genomic pathways was confirmed in studies showing that 10 nM E2 caused a 7- and 27-fold induction of reporter gene activity MCF-7 cells transfected with GAL4-ATF1 and GAL4-CREB fusion proteins and a construct containing five tandem GAL4 response elements. Thus, LDH upregulation by E2 is due to non-genomic activation of the cAMP-PKA pathway. (Supported by NIH ES09253 and ES09106)

1261 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) INDUCES PLASMINOGEN ACTIVATOR INHIBITOR-1 THROUGH AN ARYL HYDROCARBON RECEPTOR-MEDIATED PATHWAY IN A MOUSE HEPATOMA CELL LINE.

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2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), an ubiquitous environmental pollutant, elicits a variety of toxicities and is a well-known carcinogen. TCDD alters the expression of many genes including CYP1A1/2, CYP1B1, glutathione S-transferase Ya, aldehyde-3-dehydrogenase, NAD(P)H:quinone oxidoreductase, TGF-alpha, TGF-beta, plasminogen activator inhibitor-2 and interleukin-1beta. The present study investigated the effect of TCDD on plasminogen activator inhibitor-1 (PAI-1) in a mouse hepatoma cell line (Hepa1c1c7). Based on Western and Northern blots, TCDD induced dose- (0, 0.1, 1 & 10 nM) and time- (0, 3, 6, 12, 24 & 48 h) dependently PAI-1 in Hepa1c1c7 cells. However, TCDD did not induce PAI-1 in the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (Arnt)-deficient mutants derived from Hepa1c1c7 cells, indicating a functional role of the AhR-Arnt complex in this effect. Transfection with PAI-1 promoter resulted in increased PAI-1 promoter activity in Hepa1c1c7 cells treated with TCDD, but no such effect occurred in the AhR or Arnt-deficient cell lines, implying involvement of the AhR and Arnt proteins. In addition, alpha-naphthoflavone and phenanthroline, two AhR antagonists, blocked the enhancing effect of TCDD on PAI-1 promoter-coupled luciferase activity in Hepa1c1c7 cells. The results revealed that the induction of PAI-1 by TCDD occurs in the same dose range as other known TCDD-induced AhR-mediated signal transductions, implicating PAI-1 as the most recently found gene induced by TCDD.

1262 HYPOXIA INDUCES PROTEASOME-DEPENDENT DEGRADATION OF ESTROGEN RECEPTOR αA (ERα) IN HUMAN BREAST CANCER CELLS.

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Cell growth under hypoxic conditions induces a cascade of responses linked to changes in enzymes responsible for metabolic adaptation for growth and survival under low oxygen conditions. Hypoxic conditions in tumors can lead to a more clinically aggressive phenotype and, therefore, we have investigated the effects of reduced oxygen levels on key regulatory genes/proteins in estrogen receptor α (ERα)-positive ZR-75 human breast cancer cells. ZR-75 cells were maintained in an atmosphere of 21% (normoxia) or 1% (hypoxia) oxygen or grown in the presence of 0-500 μM cobaltous chloride to approximate hypoxic conditions. Western blot analysis of whole cell lysates from ZR-75 cells grown under normoxia or hypoxia for up to 24 hr showed that levels of several nuclear transcription factors, including Sp1, Sp3, hypoxia inducible factor 1β (HIF1β or Arnt) and the aryl hydrocarbon receptor (AhR), were relatively unchanged. In contrast, HIF1α protein was not detected under normotic conditions, but accumulated to very high levels between 3 - 24 hr after exposure of cells to 500 μM cobaltous chloride or 1% O_2 treatment. This increase in HIF1α was due to inhibition of proteosome-dependent degradation of HIF1α under normoxic conditions. In contrast, growth of ZR-75 cells in 500 μM cobaltous chloride or 1% oxygen resulted in > 70% degradation of ERα protein and, in time course studies, this response was observed after growth for > 3 hr in hypoxic conditions. Treatment with the proteasome inhibitor MG132 did not affect levels of ERα protein in cells maintained in 21% oxygen, whereas hypoxia-induced degradation of ERα protein was reversed by the proteasome inhibitor. The role of estrogens and antiestrogens on ERα protein and hormone-induced transactivation is complex and dependent, in part, on "optimal" expression of ERα protein. (Supported by NIH ES04176 and ES09106)

1263 NOVEL MECHANISM OF TCDD TOXICITY: SEQUESTRATION OF ARNT FROM AN ENDOGENOUS ROLE IN P53 GENE INDUCTION.

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Most of the toxic and carcinogenic actions of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD), a ubiquitous environmental contaminant, are mediated by its ability to bind and activate the aryl hydrocarbon receptor (AhR). The physiological effects of TCDD exposure include altered drug metabolism, disruption of endocrine signaling, and tissue-specific alteration of cellular processes including proliferation, differentiation, and apoptosis. Indeed, AhR directly mediates the gene induction of several xenobiotic-metabolizing enzymes *via* ineracting with its DNA binding partner, ARNT; however, the mechanisms by which AhR mediates many of the TCDD effects is poorly understood. One mechanism by which the AhR may mediate TCDD toxicity includes the sequestering of ARNT from an endogenous role. ARNT is a promiscuous nuclear transcription factor that not only homodimerizes but also dimerizes to transcription factors of the hypoxia signaling pathway (HIF-1a) and neurogenesis (Sim). To investigate the putative role of ARNT in modulating other signaling pathways, we performed transient transfection assays using a reporter gene regulated by the P53 promoter and c-myc and ARNT expression plasmids. In addition to our discovery that ARNT is able to enhance c-myc induction of the P53 gene, we have also discovered that this occurs independent of DNA binding by ARNT. In another approach, we have analyzed endogenous P53 mRNA levels within stably transformed MCF7 cells that over-express ARNT, c-myc or both. Our data indicates that TCDD toxicity may be mediated by activation of AhR, which sequester ARNT, impairs the induction of P53, and accelerates cell cycle progression and/or inhibits apoptosis.

1264 INTERACTION OF AH RECEPTOR WITH PHENOLIC ANTIOXIDANT SIGNAL TRANSDUCTION.

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The aryl hydrocarbon receptor (AhR) mediates a spectrum of adaptive and toxic responses to the environmental contaminant TCDD and related halogenated aromatic hydrocarbons. AhR may also play a role(s) in development, growth, and differentiation of tissues in the absence of an exogenous ligand. The broad range of TCDD toxicity and AhR function suggest that the mechanism of AhR action involves multiple signaling mechanisms. In this study, we examined the interaction of AhR with phenolic antioxidant signal transduction, which involves oxidative signaling. Phenolic antioxidants, such as tert-butylhydroquinone (tBHQ), hydroquinone (HQ), or catechol, induce the expression of CYP1A1 in mouse hepa1c1c7 cells; the induction is enhanced by inhibition of protein synthesis by cycloheximide (CHX) (termed superinduction). Induction by the antioxidants is both concentration and time-dependent. Furthermore, the induction and superinduction require AhR and Arnt, as they are absent in AhR or Arnt defective variant cells, and are mediated through the DRE-containing enhancer of CYP1A1, as they are reconstituted in a CYP1A1 enhancer-luciferase reporter expression system. These findings demonstrate that phenolic antioxidants can activate AhR-mediated gene transcription and suggest interaction of AhR with antioxidant mediated oxidative signaling.

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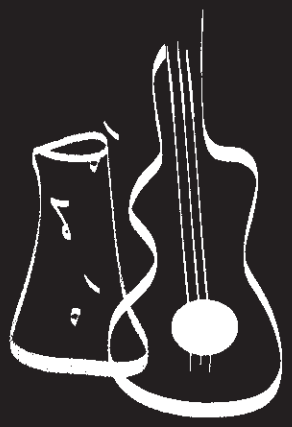

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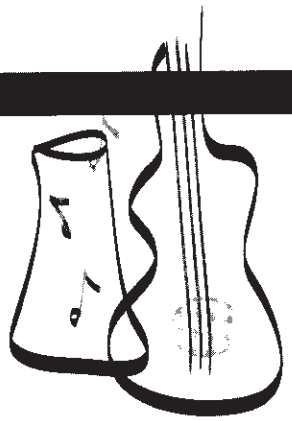

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