Species-Specific Antagonism of Ah Receptor Action by 2,2',5,5'-Tetrachlorobiphenyl.

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INTRODUCTION

Halogenated aromatic hydrocarbons (HAHs), such as the polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls, represent a class of widespread environmental contaminants. Exposure to specific HAHs results in a wide variety of species- and tissue-specific toxic and biological effects, including: birth defects, immunotoxicity, lethality, tumor promotion and enzyme induction (1,2). The induction of cytochrome P450IA1 has been utilized as the model system to examine the mechanism of action of these chemicals (3). Induction of cytochrome P450IA1 HAHs is mediated by a soluble intracellular receptor (the Ah receptor (AhR) complex) to which these chemicals bind specifically and with high affinity (1,2). Following ligand (HAH) binding, the AhR complex is transformed into a form which binds to DNA with high affinity and subsequently accumulates within the nucleus (1-4). The binding of transformed HAH:AhR complexes to a specific DNA recognition site (dioxin responsive element (DRE)) adjacent to the P450IA1 gene is necessary for its transcription activation (4,5).

Significant species- and tissue-differences in HAH responsiveness (particularly to that of the prototypical and most potent member of this class of chemicals, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD,dioxin)) have been observed (1,2). Although the AhR appears to mediate these responses in all species, species differences in the biochemical and physiochemical properties of the AhR as well as in the affinity and specificity of ligand binding have been observed (6,7). We have recently observed functional antagonism of TCDD-inducible, AhR-mediated induction of P450IA1 in mouse hepatoma cells by several "nonAhR" polychlorinated biphenyls (PCBs) (See Arts et al., Dioxin 93 Abstracts). This observation is of particular importance, given that HAHs exist as complex mixtures and that interactions between chemicals within the mixture could differentially affect the overall biological/toxicological potency of the mixture to various species. Here we have examined mechanism of this antagonism by examining the effect

of TCB on AhR functionality (AhR transformation, DNA binding and transcriptional activation).

MATERIALS AND METHODS

Preparation of Cytosol: Hepatic cytosol from male Hartley guinea pigs (250-300g, Michigan Department of Public Health, Lansing, MI USA), and male Sprague-Dawley rats (200g, Charles River Laboratories, Wilmington, DE USA)) and from mouse hepatoma (hepa1c1c7) cells (hepa1) was prepared as previously described (7,8).

Expression Assay: Hepa1 cells which have been stably transfected with the HAH-inducible reporter plasmid pGudluc1.1 (See Denison et al., Dioxin 93 Abstracts) were incubated with the indicated concentrations of TCB and/or TCDD for 24 h. After incubation, cells were lysed and LUC activity of the cleared lysate was determined using the Promega luciferase assay system.

Gel Retardation Analysis: A complementary pair of synthetic DNA fragments containing the sequence 5'-GATCTGGCTCTTCTCACGCAACTCCG-3' and 5'-GATCCGGA-GTTGCGTGAGAAGAGCCA-3' (corresponding to the 20-bp AhR binding site of DRE3 (5) and designated here as the DRE oligonucleotide) were radiolabeled with [^{32}P]ATP as previously described (4). Gel retardation analysis was carried out as previously described (4,5) and the amount of specific TCDD:AhR:DRE complex was determined, following autoradiography of dried gels, by excision of the specific protein-DNA complex from the dried gel and measurement of the radioactivity in the complex.

RESULTS

Incubation of pGudluc1.1-stabily transfected mouse hepa1 cells with increasing concentrations of TCB resulted in a dose-dependent decrease in TCDD-inducible LUC activity (Fig. 1), suggestive of antagonism of AhR action. TCB also produced a dose-dependent decrease in basal LUC activity. We have observed that the transfected cells have an elevated level of basal LUC activity and, although it is not clear what is responsible for this basal activity, TCB inhibit/antagonize it. The background LUC activity could result from the presence of an inducer in the medium, an endogenous inducer produced by the cells and/or integration of the vector into the genome at a site(s) that result in constitutive expression of the LUC gene.

Incubation of hepa1 cytosol with TCDD results in an inducible protein-DNA complex which represent the TCDD:AhR complex bound to the DRE (Fig. 1) and the addition of TCB to this incubation decreased the amount of inducible protein-DNA complex. Since previous studies with α-napthoflavone, an AhR antagonist, can produce the results observed here (relative to induction and AhR DNA binding), we interpret our data to mean that TCB can act as an AhR antagonist. The TCB-dependent decrease in protein-DNA complex observed in the absence of TCDD is due to elimination of the background protein-DNA complex that is commonly observed in the absence of added inducer (9).

In order to determine if species differences in TCB action exist, we

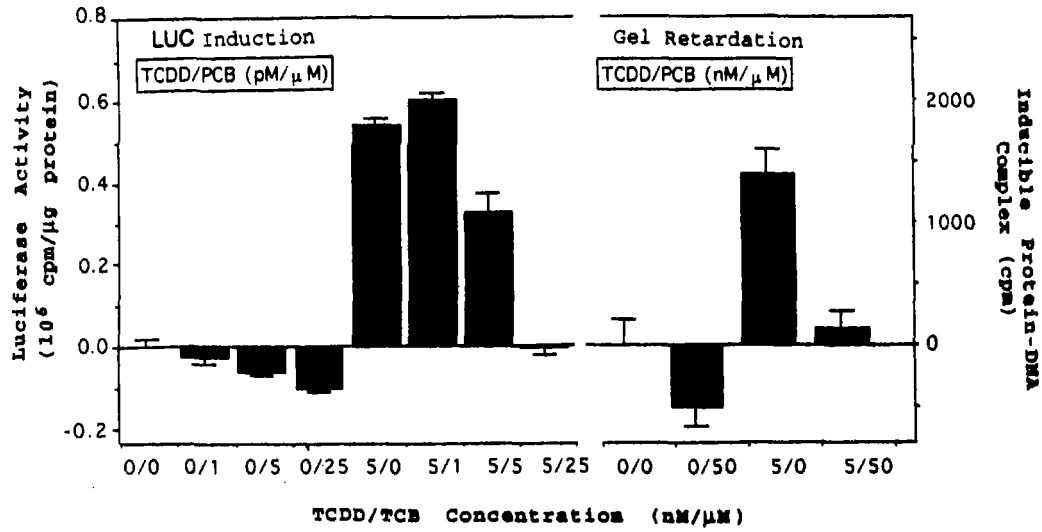

Figure 1. Effect of TCB on TCDD-inducible LUC expression and DNA binding activity of the AhR complex.

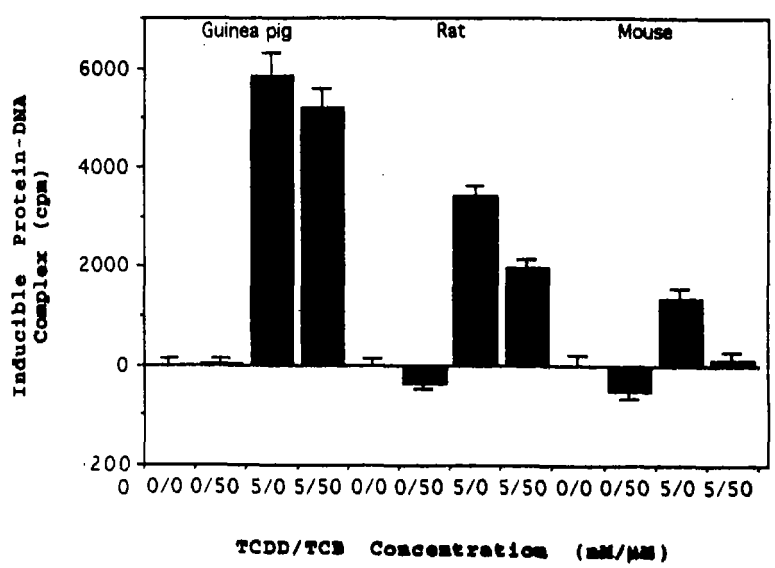

Figure 2. Species differences in the ability of TCB to affect the DNA binding activity of the AhR complex.

examined the effect of TCB on TCDD:AhR:DRE complex formation using guinea pig and rat cytosolic AhR. Although TCB inhibits inducible TCDD:AhR:DRE complex formation using hepa1 mouse cytosol (by about 90%), it had no apparent effect on complex formation using guinea pig hepatic cytosol; rat hepatic AhR complex was inhibited by about 40%. Thus, TCB exhibits species-dependent antagonistic effects (presumably related to its ability to bind to the AhR from each species) and suggests that a given chemical mixture could exert significantly different effects in different species.

CONCLUSIONS

Our results demonstrate a significant species difference in the ability of a previously documented "nonAhR" ligand to act as an AhR antagonist. These results raise some interesting questions regarding analysis of the biological effects of complex mixtures are examined. The presence of high levels of TCB in a complex mixture could affect its biological activity in guinea pig significantly less than that in rat or mouse. Which of these species might be comparable to effects seen on human AhR remains to be determined. We are currently investigating the molecular mechanism of antagonism of TCB and other "nonAhR" PCBs in greater detail.

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