Synergistic Interactions of Polynuclear Aromatic Hydrocarbons: Induction of CypIa-1 in B6C3/F1 Mice

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Abstract

The induction of hepatic microsomal ethoxyresorufin O-deethylase (EROD) activities by benzo(a)pyrene (B(a)P) and complex mixtures of polynuclear aromatic hydrocarbons (PAHs) associated with manufactured gas plant residues were determined in B6C3/F1 mice. Based on the B(a)P content and the levels of other aryl hydrocarbon (Ah) agonists in the PAH mixtures, the potencies of several PAH mixtures were 2 to 3 orders of magnitude higher than expected for the induction of EROD activity and for other Ah receptor-mediated responses. Moreover, in studies which utilized reconstituted mixtures of individual PAHs in which the relative concentrations of aryl hydrocarbon receptor agonists were <5% of the total mixture, it was shown that there was a significant synergistic induction of EROD activity in B6C3/F1 mice and in mammalian cells in culture. The contribution of the different structural classes of PAHs (*i.e.* 2, 3, and ≥ 4 rings) to the apparent synergistic induction response and the possible influence of this effect on the carcinogenic potency of PAH mixtures will be discussed.

Introduction

Polynuclear Aromatic Hydrocarbons (PAHs) elicit a diverse spectrum of biochemical and toxic responses in laboratory animals and mammalian cells in culture. Some of these responses are mediated through the Ah receptor, an intracellular protein which binds TCDD and related PAHs with high affinity. The proposed mechanism for the induction of cytochrome p450Ia1 (CypIa1) and its associated monooxygenase activities is thought to involve specific binding of TCDD and related aryl hydrocarbons to the Ah receptor (1). Extensive molecular biology studies on the induction of CypIa1 gene expression by PAHs have shown that the liganded cytosolic Ah receptor complex undergoes transformation and nuclear translocation, in which the resulting Ah receptor complex binds to cis-acting genomic sequences (dioxin responsive elements, DREs). This interaction results in enhanced gene transcription (2-5).

PAHs occur as complex mixtures in environmental samples and industrial effluents. In this study, manufactured gas plant (MGP) residues were investigated as inducers of hepatic microsomal ethoxyresorufin-O-deethylase (EROD) activity, and their effects on transformation of the cytosolic Ah receptor were determined using a gel retardation assay. Benz(a)pyrene, the most potent PAH in the MGP, was used for comparison studies.

Materials and Methods

Chemicals and Biochemicals. The MGP residue is a complex mixture of PAHs and related compounds produced as a by-product of the coal gasification process. NADP, NADPH, bovine serum albumin, and compounds required for the reconstitution mixture were purchased from Sigma Chemical Co. (St. Louis, MO). MCF-7 human breast cancer cells were purchased from ATCC (Rockville, MD).

Treatment of Animals. Male Long-Evans rats (21 days old, ~ 100 g) were obtained from Harlan Laboratories (Houston, TX) and housed two per cage in plastic cages with hardwood bedding. Rats were maintained on a diurnal cycle of 12 hr light/dark with free access to food and water. Rats were allowed to acclimate 4 days prior to CO_2 asphyxiation. B6C3/F1 mice were obtained from Charles River Laboratories (Boston, MA). Chemicals dissolved in corn oil were administered i.p. to 15 day old male pups. Mice were sacrificed by cervical dislocation 24 hr following treatment.

Preparation of Hepatic Cytosol. The animals were terminated by CO2 asphyxiation, and the livers were immediately perfused in situ via the hepatic portal vein with ice-cold HEGD buffer [25 nM hepes, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol (v/v), pH 7.6]. The excised livers were rinsed once with 10 ml of the fresh ice-cold HEGD buffer and finely minced. The minced livers were rinsed again with the buffer and homogenized using a Brinkman Homogenizer PT45/80. The homogenate was centrifuged at 10,000 g for 20 minutes at 2°C, and the lipid layer was removed by aspiration. The resulting supernatent was recentrifuged at 105,000 g for 1 hr at 2°C. Protein concentrations were measured by Lowry et al. (6) and the cytosol was stored in liquid nitrogen until use.

Induction of EROD Activity. EROD activity was determined by the method of Pohl and Fouts (7). Protein concentration were determined by the method of Lowry et al. (6).

Gel Shift Assays. A complementary pari of oligonucleotides containing the sequence 5'-GATCTGGCTCTTCTCACGCAACTCCG-3' was synthesized, purified by polyacrylamide gel electrophoresis, annealed and ^{32}P-lableled at the 5'-end using T4 polynucleotide kinase and (γ-^{32}P)ATP. DNA binding was measured using a gel shift assay according to the method of Denison and Deal (8).

Statistical Analysis. The statistical differences among different groups were determined by ANOVA in which the data are expressed as means ± standard deviations.

Results and Discussion

Manufactured gas plant residue (coal tar), MGP extract (cleaned-up coal tar), reconstituted MGP, and Benzo(a)pyrene exhibited a concentration-dependent effect on the induction of EROD activity in B6C3/F1 mice (Figs. 1-4). Treatment of rat hepatic cytosol with MGP, MGP extract, reconstituted MGP, and B(a)P resulted in a

concentration-dependent transformation of the rat cytosolic Ah receptor as determined by gel retardation assays (Fig. 5). Analysis of the MGP residue by GC/MS showed that the individual PAHs which exhibit Ah receptor agonist activity comprise less than 5% of the total mixture. However, the different structural classes of PAHs, when in combination, elicit a more potent EROD induction response than the individual components by themselves. The 25 to 30 fold increase in EROD activity by the reconstituted mixture suggests that synergistic effects are due to the combination of the individual PAH components. However, the reasons for these non-additive interactions have not yet been determined.

References

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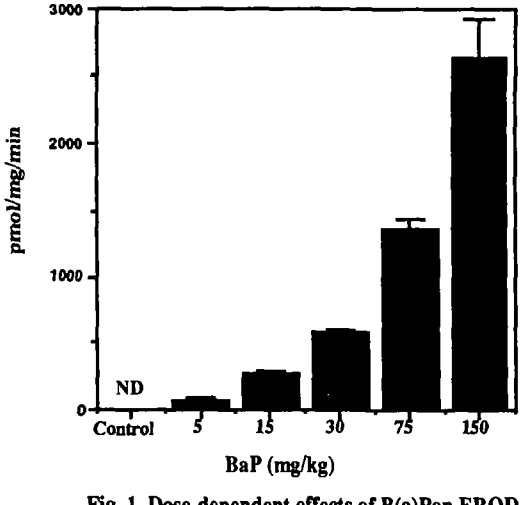

Fig. 1 Dose-dependent effects of B(a)Pon EROD induction in B6C3/F1 mice

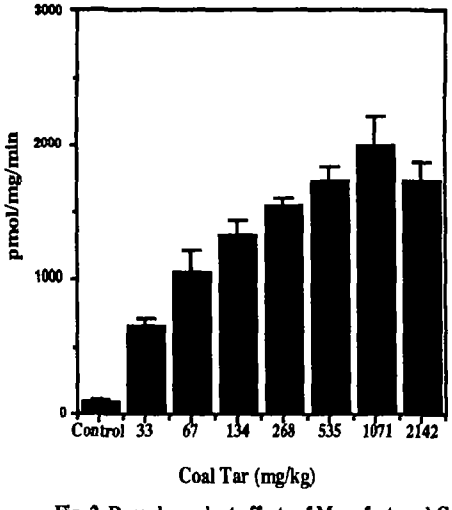

Fig. 2 Dose-dependent effects of Manufactured Gas Plant Residue (Coal Tar) on EROD induction in B6C3/F1 mice

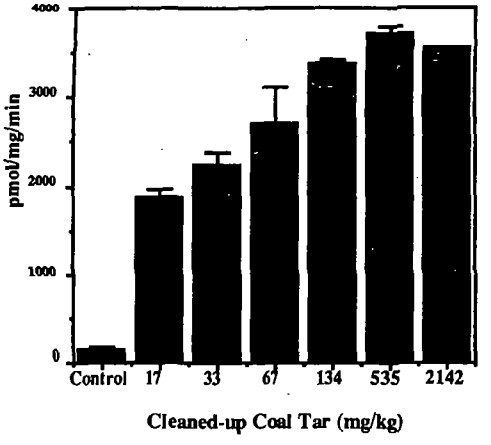

Fig. 3 Dose-dependent effects of hexane treated Manufactured Gas Plant Residue (Cleaned-up Coal Tar) on EROD induction in B6C3/F1 Mice

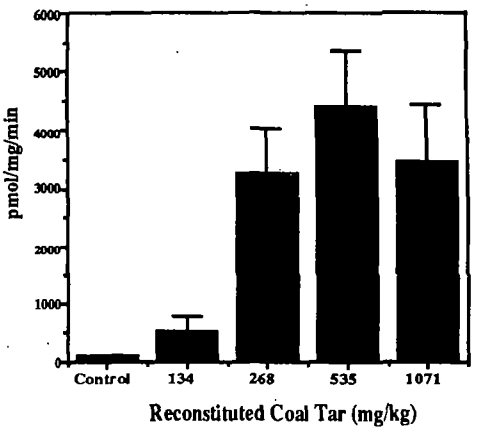

Fig. 4 Dose-dependent effects of Reconstituted Manufactured Gas Plant Residue (Reconstituted Coal Tar) on EROD induction in B6C3/F1 mice

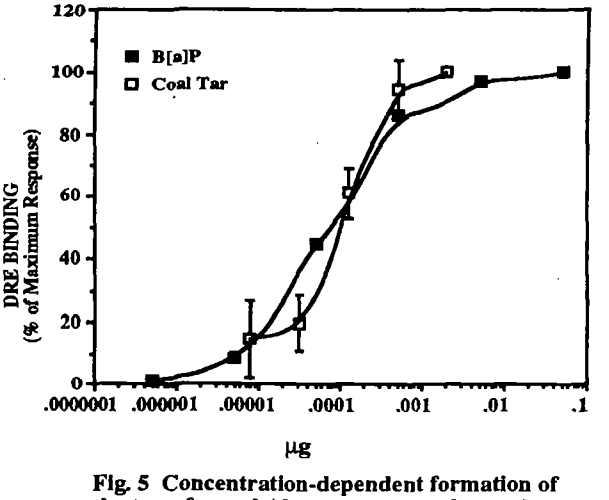

Fig. 5 Concentration-dependent formation of the transformed Ah receptor complex as the determined by the gel retardation assay