0026-895X/89/010121-07$02.00/0

MOLECULAR PHARMACOLOGY, 36:121-127

Stimulation of *In Vivo* Hepatic Uptake and *In Vitro* Hepatic Binding of [^{125}I]2-Iodo-3,7,8-trichlorodibenzo-*p*-dioxin by the Administration of Agonists for the Ah Receptor

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Received November 28, 1988; Accepted April 11, 1989

SUMMARY

[^{125}I]2-Iodo-3,7,8-trichlorodibenzo-*p*-dioxin ([^{125}I]Cl_3D*p*D), a radiolabeled, isosteric, analogue of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), was synthesized and used to study *in vivo* tissue localization and *in vivo* tissue binding. Twenty-four hours after the administration of a tracer dose (1×10^{-10} mol/kg) of [^{125}I]Cl_3D*p*D to C57BL/6J mice, the hepatic concentration of radioactivity was 1–2% of the administered dose, whereas in mice pretreated with TCDD (1×10^{-7} mol/kg), the hepatic accumulation of radiolabel was 25–30% of that administered. Liver homogenate from TCDD-treated mice bound 4 to 10 times more [^{125}I]Cl_3D*p*D than homogenate from control mice. The enhancement of *in vivo* uptake and *in vitro* tissue binding of [^{125}I]Cl_3D*p*D by TCDD administration was confined to liver and was not observed in other tissues examined, kidney, lung, spleen, small intestines, and muscle. The administration of TCDD to C57BL/6J mice produces dose-related stimulation of *in vivo* hepatic uptake of [^{125}I]Cl_3D*p*D, binding of radioligand to liver homogenate, and hepatic aryl hydrocarbon hydroxylase activity, with the dose for half-maximal stimulation, ED_{50}, varying from 1.5 to 4.0 $\times 10^{-9}$ mol/kg. In congenic C57BL/6J (Ah^d/Ah^d) mice, which express the lower affinity Ah receptor, the ED_{50} values for all three responses were shifted to ~10-fold higher doses. 3,3′,4,4′,5,5′-Hexabromobiphenyl, a weak agonist for the Ah receptor produced a dose-related stimulation of these three responses in C57BL/6J mice (ED_{50} values of $\sim 5 \times 10^{-7}$ mol/kg), but was without effect in C57BL/6J (Ah^d/Ah^d) mice. Stimulation of *in vivo* hepatic uptake and *in vitro* liver homogenate binding of [^{125}I]Cl_3D*p*D was produced by administration of Ah agonists, such as 2,3,7,8-tetrachlorodibenzofuran and β-naphthoflavone, but inactive congeners and other compounds that do not act via the Ah receptor, e.g., phenobarbital and pregnenolone-16α-carbonitrile, did not evoke these effects. Thus, TCDD and other Ah agonists act through the Ah receptor to increase a liver binding species that increases the hepatic uptake of [^{125}I]Cl_3D*p*D *in vivo* and binding of this radioligand to liver homogenate *in vitro*.

TCDD, a trace contaminant of chlorophenol synthesis, has received much attention because of its toxic potency (and hence potential health hazard) and its interesting mechanism of action (1–3). TCDD serves as the prototype of a large group of halogenated aromatic hydrocarbons (certain chlorinated and brominated dibenzo-*p*-dioxin, dibenzofuran, biphenyl, and azo(*xy*)benzene congeners), which are all approximate isostereomers and which produce a similar and characteristic pattern of biochemical and morphological responses. These compounds are thought to exert most, if not all, of their biological effects by stereospecific, high affinity binding to a soluble protein, the Ah receptor, and the coordinate gene expression initiated by the receptor-ligand complex (2). Studies on the gene for cytochrome P_1-450 suggest that the TCDD-Ah receptor complex binds to DNA regulatory elements upstream from the start site for the structural gene and initiates transcription (3). Thus, the Ah receptor bears many similarities to the steroid hormone receptors, and it is postulated to be a member of the *erb-A* family of genes (4).

The pharmacokinetic behavior of TCDD has been investigated in a number of animal species (5). In most species, TCDD is concentrated in the liver and adipose tissue, slowly metabolized, and eliminated largely as polar metabolites in urine and feces, with a relative long whole body half-life (ranging from 10 to 30 days in laboratory rodents). Following the administration of [^{3}H]TCDD (~10^{-9} mol/kg) to rats and mice, the highest tissue concentration is in liver, equivalent to 20–40% of the total dose (5–8). Upon subcellular fractionation of the liver, the highest concentrations of radiolabel were associated with the

This work was supported in part by the National Institute of Environmental Health Science Grant ES-01884 and the National Cancer Institute Core Grant 07175.

ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; [^{125}I]Cl_3D*p*D, [^{125}I]2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin; TCDBF, 2,3,7,8-tetrachlorodibenzofuran; MOPS, 3-[*N*-morpholino]propanesulfonic acid; MES, 2-[*N*-morpholino]ethanesulfonic acid; DMSO, dimethylsulfoxide; TCPOBOP, 1,4,-bis[2-(3,5-dichloropyridyloxy)]benzene; BSA, bovine serum albumin; AHH, aryl hydrocarbon hydroxylase.

microsomal fraction (5). This hepatic localization of TCDD is in contrast to many compounds of comparable lipophilicity, which are more concentrated in adipose tissue.

The following evidence suggested that the hepatic localization of TCDD was due to sequestration by binding to the Ah receptor: (a) after administration of TCDD (~6×10^{-9} mol/kg), a high hepatic concentration was found in C57BL/6J mice (which have a high affinity Ah receptor), a low hepatic concentration in DBA/2J mice (which have a low affinity receptor), and an intermediate concentration in the heterozygote B6D2F_1 mice; and (b) administration of a congener,2,3-dichlorodibenzo-*p*-dioxin, which is not a receptor agonist, resulted in low hepatic concentrations, which were equivalent in C57BL/6J, DBA/2J, and B6D2F_1 mice (6). However, this interpretation is quantitatively implausible, because the TCDD concentration in liver was 10 to 100 times greater than the hepatic Ah receptor concentration. Teitelbaum (9) found that treatment of mice with TCDD or other agonists for the Ah receptor produced an increased hepatic uptake of a subsequent tracer dose of [^{3}H]TCDD *in vivo*, and liver homogenate from treated mice bound more [^{3}H]TCDD *in vitro*. Thus, TCDD treatment enhances a hepatic binding species, distinct from the Ah receptor, that is responsible for hepatic localization of the compound.

Teitelbaum's studies were published in a thesis (9). In this report and the accompanying paper, we have repeated and extended these observations with newer methodology that permits a more precise biochemical examination of the problem.

We have synthesized [^{125}I]Cl_3D*p*D as a radiolabeled analogue of TCDD, which differs by the substitution of one iodine for one chlorine atom. [^{125}I]Cl_3D*p*D has several advantages over [^{3}H]TCDD, a higher specific activity and counting efficiency and the γ-emission that permits measurement of the rate of drug elimination by noninvasive whole body counting.[1]

In this report we examine the effect of TCDD administration on the *in vivo* hepatic uptake of a tracer dose of [^{125}I]Cl_3D*p*D and on the *in vitro* binding of radioligand to liver homogenate.

Materials and Methods

Bovine serum albumin (A-4503), MOPS, MES, glycine, and Tris were purchased from Sigma Chemical Co. (St. Louis, MO), GF/A filters (2.4-cm diameter) were purchased from Whatman, Inc. (Clifton, NJ). TCDD was a gift from Dow Chemical Co. (Midland, MI), and TCDBF was a gift from Dr. David Firestone, Food and Drug Administration (Washington, DC). Zoxazolamine was a gift of McNeil Pharmaceutical (Springhouse, PA).

Synthesis of [^{125}I]Cl_3D*p*D. Fifty micrograms (0.165 μmol) of 2-amino-3,7,8-trichlorodibenzo-*p*-dioxin (9) dissolved in benzene, were dried down in a 1-ml conical vial that contained a microstirring bar and the vial was cooled in an ice bath at 0°. Thirty microliters of ice-cold 50% aqueous sulfuric acid were added and the reaction was stirred for 10 min, followed by the addition of 6 μl of an aqueous solution of $NaNO_2$ (10 mg/ml) and stirring for 30 min at 0°. Excess nitrite was consumed by the addition of 2 μl of an aqueous urea solution (100 mg/ml) with 2 min of stirring. Five millicuries of carrier-free Na^{125}I (New England Nuclear NEZ-033L; ≥350 mCi/ml in 6 mM NaOH; ≤15 μl of solution), in the manufacturer's sealed vial, was cooled in an ice bath, and 9 times the molar amount of unlabeled NaI (20.68 nmol; 3.1 μg) in 5 μl of 6 mM NaOH was added and stirred. One half the volume of the first vial (19 μl), containing 2-diazonium 3,7,8-trichlorodibenzo-*p*-dioxin, was added to the sealed vial that contained Na^{125}I plus NaI and the reaction was incubated at 20° for 2 hr. The reaction was terminated by the addition of 200 μl of an aqueous solution that contained 1.35 N NaOH, 0.1 M sodium borate, and 400 μg of $Na_2S_2O_5$, and the reaction product was extracted into 200 μl of chloroform. The chloroform extract was dried down, with a stream of nitrogen, under an inverted funnel in series with a charcoal trap hooked to a vacuum line in a chemical hood. The product was dissolved in 25 μl of *p*-dioxane and purified by high pressure liquid chromatography. In a nonradioactive trial, 114 mg of 2-amino-3,7,8-trichlorodibenzo-*p*-dioxin was converted to the 2-iodo compound by the above procedure. The crude product was recrystallized six times from hot chloroform to give 57 mg (37% yield) of pure 2-iodo-3,7,8-trichlorobenzo-*p*-dioxin, m.p. 257–259° (decomposed). Calculated for $C_{12}H_4Cl_3IO_2$: C, 34.84; H, 0.97. Found: C, 35.06; H, 1.02. On an Altex ODS column (0.46 × 25 cm) with isocratic elution using methanol/H_2O (92:8) at a flow rate of 1 ml/min, authentic unlabeled 2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin eluted at 24 min, with a UV absorption maximum at 242 nm. The radiolabeled product with the same retention time and UV maximum was collected, and had a maximum initial specific activity of 272 Ci/mmol (1:9 dilution of the carrier-free ^{125}I); the yield was usually ~15% or 750 μCi of ^{125}I incorporated. The product was stored at 1×10^8 cpm/ml in methanol and was protected from light.

The radiosynthesis was performed in a special room equipped with an excellent laminar flow chemical hood, with sealed vials, and was monitored with a portable γ-scintillation counter.

Animals. C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were bred and maintained in our facility. Congenic C57BL/6J (Ah^d/Ah^d) mice are homozygous for the Ah^d allele, which encodes the low affinity Ah receptor (6). These mice were originally bred by Dr. Daniel Nebert, National Institute of Child Health and Human Development (Bethesda, MD) by selecting mice in the F_2 generation of a C57BL/6N × DBA/2N cross, maintained by a backcross (to C57BL/6)/intercross (F_1) breeding system and were provided to us in the 13th generation [B6N·D2N(Ah^d)$N_{13}F_{13}$]. We bred them using the same backcross/intercross system for 14 generations into C57BL/6J mice ($N_{14}F_{14}$) and then for two generations by homozygous (Ah^d/Ah^d) matings (see Figs. 7, 8, and 9). The mice were phenotyped in the even generations by failure of β-naphthoflavone administration to shorten zoxalamine paralysis times (10). All mice were maintained in plastic cages on hard wood chip or corn cob bedding, with mouse chow (Wayne Mouse Rodent Blox; Wayne Pet Food Division, Continental Grain Co., Chicago, IL) and water *ad libitum*, with a 12-h light/dark diurnal cycle at constant temperature and humidity.

Sprague-Dawley rats, Syrian golden hamsters, and Hartley guinea pigs were obtained from the Harlan Sprague-Dawley Co. (Madison, WI).

Standard dosage regimens. C57BL/6J mice were routinely treated with TCDD (1 or 3×10^{-7} mol/kg dissolved in *p*-dioxane, 0.3 ml/kg) or *p*-dioxane alone (control mice) by intraperitoneal injection, using 10-μl microsyringes (Hamilton, Reno, NV). For the measurement of *in vitro* binding of radioligand or hepatic AHH activity, the animals were killed 48 hr after dosing. For the determination of *in vivo* hepatic uptake of radiolabel, 48 hr after dosing the mice were treated with [^{125}I]Cl_3D*p*D (1×10^{-10} mol/kg; initial specific activity ~272 Ci/mmol, or ~9.6×10^5 dpm/20-g mouse) dissolved in *p*-dioxane (0.3 ml/kg), intraperitoneally, and were killed 24 hr later.

Hepatic uptake of [^{125}I]Cl_3D*p*D. Twenty-four hours after the administration of [^{125}I]Cl_3D*p*D, the mice were killed, the livers were removed, and the radioactivity in the entire liver was determined by γ-scintillation counting (Minaxi 5000; United Technologies-Packard, Des Plaines, IL). The raw cpm were corrected for background and counting efficiency, and hepatic radioactivity was expressed as the fraction of the dose of [^{125}I]Cl_3D*p*D that was administered to each mouse.

Hepatic AHH activity. The livers were weighed, homogenized in 9 volumes of MEN buffer (25 mM MOPS, 1 mM EDTA, 0.02% NaN_3, pH 7.5 at 20°), and centrifuged at 10,000 × *g* for 20 min at 4°. AHH activity was determined in the 10,000 × *g* supernatnat fraction as

[1] A. Poland, P. Geiger, and E. Glover. Manuscript in preparation.

previously described (11), with calibration of the product fluorescence with an authentic standard of 3-hydroxybenzo(*a*)pyrene. Activity is expressed as units; 1 unit equals 1 pmol of 3-hydroxybenzo(*a*)pyrene formed/min/supernatant fraction equivalent to 1 mg wet weight of liver.

Standard binding assay. A solution of BSA (Sigma No. A-4503), 20 mg/ml in MEN buffer, was prepared, filtered through GF/A glass filters, and stored for up to 2 weeks at 4°. Mouse liver was homogenized in 9 volumes of ice-cold MEN buffer, filtered through 100-μm nylon mesh, and diluted to 0.5 mg wet weight of liver/ml. The liver homogenate could be stored at −80° for several weeks without a change in binding activity. From a stock solution of [^{125}I]Cl_3DpD in DMSO, an aliquot was added to the buffered BSA solution to give a concentration of 0.3 pmol of radioligand (~1.44×10^5 dpm) and 2.5 μl of DMSO in 0.5 ml. A 0.5-ml aliquot of the diluted liver homogenate and an equal volume of the radioligand-BSA solution were mixed and incubated for 45 min at 20°. The binding reaction was terminated by pipetting the mixture onto a GFA/A glass filter (2–4-cm diameter) that was fitted to a filtration manifold (Hoefer Scientific Instruments, San Francisco, CA; Model FH 224V, with vacuum setting at 2.0), separating the filter-trapped bound radioligand, and washing the filter three times with 1.5 ml of ice-cold MEN buffer that contained 1 mg of BSA/ml. The filter paper was placed in a 10 × 75 mm test tube, and the radioactivity was quantified by γ-scintillation counting.

Results

After the administration of [^{125}I]Cl_3DpD (1×10^{-10} mol/kg) to C57BL/6J mice, the radioactivity in the liver reaches a maximum at ~3 hr and then declines to a plateau concentration, equivalent to ~1.5% of the administered dose, by 24 hr (Fig. 1). Pretreatment of mice with TCDD (1×10^{-7} mol/kg) produced a marked increase in the hepatic uptake of radioactivity, equivalent to 26 to 29% of the administered dose of [^{125}I]Cl_3DpD. As will be shown below, the liver homogenate from TCDD-treated mice binds [^{125}I]Cl_3DpD *in vitro* to a greater extent than does homogenate from control (vehicle-treated) mice. In this report we first characterize the *in vitro* binding assay, and then examine the effect of drug administration on the *in vivo* uptake and *in vitro* binding of [^{125}I]Cl_3DpD.

***In vitro* binding assay.** A dilute suspension of liver homogenate in buffer was incubated with BSA and radioligand, and the bound radioligand was separated by filtration, washed, and quantified (see Materials and Methods). The assay was devised to address several properties of the ligand and the binding moiety [which are examined in greater detail in the accompanying paper (12)], as follows: (a) the major binding species is in the membrane fraction and, hence, is collected by filtration; (b) the pool of binding sites is very large, and it is necessary to greatly dilute liver homogenate to achieve appreciable fractional saturation; and (c) in light of the low protein (homogenate) concentration and the limited aqueous solubility and hydrophobicity of the radioligand, it is necessary to add an

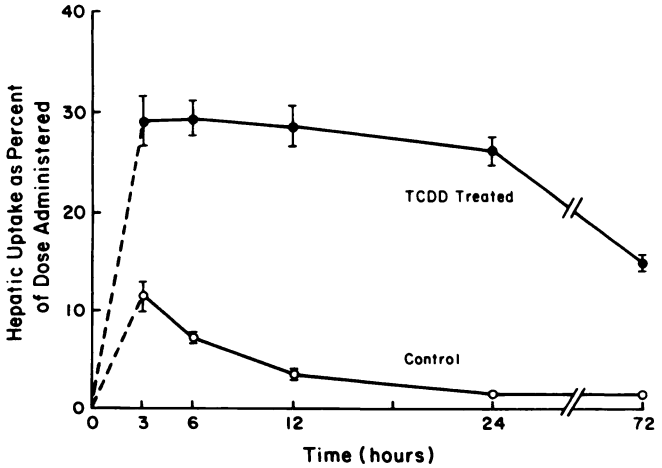

Fig. 1. Time course of the hepatic concentration of [^{125}I]Cl_3DpD in control and TCDD-treated mice. C57BL/6J female mice (7–9 weeks old) were treated with TCDD (1×10^{-7} mol/kg, dissolved in *p*-dioxane, 0.3 ml/kg) or the vehicle alone (i.e., control mice) by intraperitoneal injection, and 48 hr later all mice were treated with [^{125}I]Cl_3DpD (1×10^{-10} mol/kg). The animals were killed at the indicated times after injection of the radiolabel, and the radioactivity in their livers was determined by γ-scintillation counting and expressed as the percentage of the dose administered. The graph shows the mean values ± standard error for five animals.

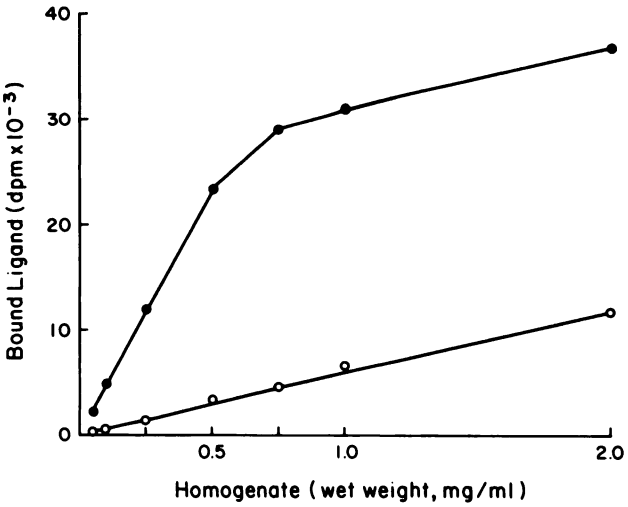

Fig. 2. [^{125}I]Cl_3DpD binding as a function of the concentration of liver homogenate. [^{125}I]Cl_3DpD binding to liver homogenate was determined under standard assay conditions as outlined in Materials and Methods. All values were corrected for ligand binding to the filters in the absence of homogenate (300 dpm). ●, TCDD-treated; ○, control (solvent-treated).

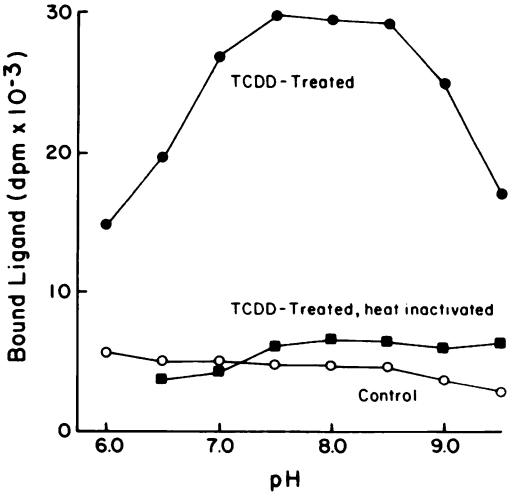

Fig. 3. [^{125}I]Cl_3DpD binding as a function of pH. [^{125}I]Cl_3DpD binding was determined by the standard assay (see Materials and Methods) except that the buffer for incubation and filter wash consisted of 10 mM concentrations of MES, MOPS, Tris, and glycine adjusted to the indicated pH. [^{125}I]Cl_3DpD binding to liver homogenate from TCDD-treated mice: ●, total bound ligand; □, heat-inactivated homogenate from TCDD-treated mice (nonspecifically bound ligand); ○, total bound ligand to homogenate from control (solvent-treated) mice.

appreciable quantity of BSA to serve as a pool of soluble, low affinity, binding sites that reduce nonspecific adsorption of the radioligand to the homogenate. In most cases data are expressed as total bound ligand.

As seen in Fig. 2, [^{125}I]Cl_3D*p*D binding is a function of homogenate concentration, linear to 0.5 mg wet weight of liver/ml for homogenate from TCDD-treated mice and to ≥2 mg wet weight of liver/ml for homogenate from control mice. In all subsequent experiments, we used a concentration of 0.25 mg of liver homogenate/ml. Total ligand binding to homogenate from TCDD-treated animals had a broad pH optimum, between pH 7.0 and 8.5 (Fig. 3). Heat treatment (60° for 10 min) of this homogenate greatly reduced ligand binding. We conclude that binding to heat-inactivated homogenate is nonspecific binding. Liver homogenate from control mice had much less total ligand binding, and this was only slightly decreased by heat treatment (data not shown), suggesting that specific binding capacity (total minus nonspecific binding) is quite small in control liver. [^{125}I]Cl_3D*p*D was added in a small volume of DMSO (≤5 μl/ml assay); concentrations of DMSO up to 10 μl/ml had minimal effect on binding (data not shown). Ligand binding is a function of temperature and time of incubation. At 20° equilibrium binding was achieved by incubation for 45 min (data not shown). BSA in the incubation mixture produced a concentration-dependent decrease in [^{125}I]Cl_3D*p*D binding to homogenate (Fig. 4). A BSA concentration of 10 mg/ml was used in all subsequent assays. The ligand-homogenate complex was collected on glass filters and washed three times with ice-cold MEN buffer that contained 1 mg/ml BSA, which produced a modest decrease in filter-trapped radiolabel.

We next examined the effects of administered compounds on the hepatic uptake of [^{125}I]Cl_3D*p*D *in vivo* and binding of this radioligand to liver homogenate *in vitro*.

Tissue localization. TCDD administration produces a large increase in the *in vivo* uptake of [^{125}I]Cl_3D*p*D in liver but not in the other tissues examined (Table 1). Similarly, TCDD treatment increased the total binding of the radioligand *in vitro* to liver homogenate substantially more than binding to homogenate from other tissues (Table 2).

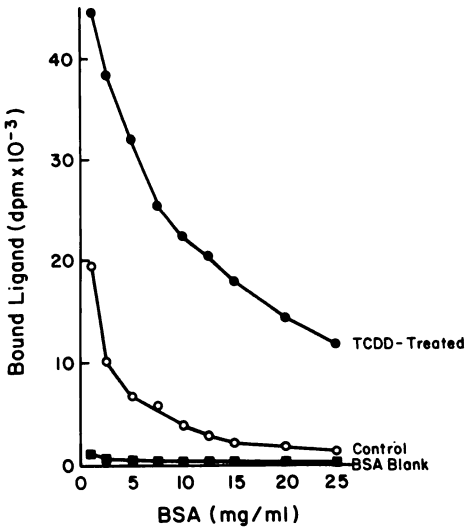

Fig. 4. Effect of the BSA concentration on ^{125}I binding. Total ligand binding was determined by the standard assay (see Materials and Methods) except the BSA concentration in the incubation mixture was varied. Total ligand binding to liver homogenate from TCDD-treated mice (●) and from control (solvent-treated) mice (○) and binding in the absence of homogenate, i.e., BSA blank (■) are shown.

TABLE 1

Tissue concentrations of radioactivity after administration of [^{125}I] Cl_3D*p*D to control and TCDD-treated mice

C57BL/6J male mice were treated with TCDD (3×10^{-7} mol/kg) in *p*-dioxane (0.3 ml/kg) or the vehicle alone, intraperitoneally, and 48 hr later all mice received [^{125}I] Cl_3D*p*D (1×10^{-10} mol/kg) intraperitoneally. The animals were killed 24 hr later, the tissue samples were weighed, and the radioactivity was quantified. Values are mean ± standard error of determinations on four animals.

Tissue	Radioactivity		
	Control	TCDD-Treated	TCDD-Treated/Control
	% of dose/gm of tissue		
Liver	1.82 ± 0.35	23.5 ± 2.5	12.9
Kidney	0.45 ± 0.15	0.42 ± 0.02	0.9
Lung	1.01 ± 0.16	0.57 ± 0.06	0.6
Spleen	0.59 ± 0.12	0.29 ± 0.08	0.5
Small intestine	0.42 ± 0.05	0.36 ± 0.04	0.9
Muscle	0.17 ± 0.03	0.09 ± 0.01	0.5

TABLE 2

***In vitro* binding of [^{125}I]Cl_3D*p*D to various tissue homogenates from TCDD-treated and control mice**

Total ligand binding to tissue homogenates from TCDD-treated and control (solvent-treated) mice was determined using standard assay conditions (see Materials and Methods). The tissue homogenate concentration was 0.5 mg/ml for liver and 1.0 mg/ml for all other tissues. Values are mean ± standard error of determinations on four animals in all cases except control muscle, where three animals were used.

Tissue	Binding		
	Control	TCDD-Treated	TCDD-Treated/Control
	dpm × 10^{-3}		
Liver	7.5 ± 0.2	38.5 ± 1.7	4.6
Kidney	7.6 ± 0.3	9.0 ± 0.1	1.2
Lung	9.8 ± 0.3	12.1 ± 0.1	1.2
Spleen	4.2 ± 0.8	3.7 ± 0.6	0.9
Small intestine	3.3 ± 0.6	4.4 ± 0.7	1.3
Fat	11.1 ± 0.7	13.9 ± 1.9	1.3
Muscle	8.0 ± 0.6	5.3 ± 0.2	0.7

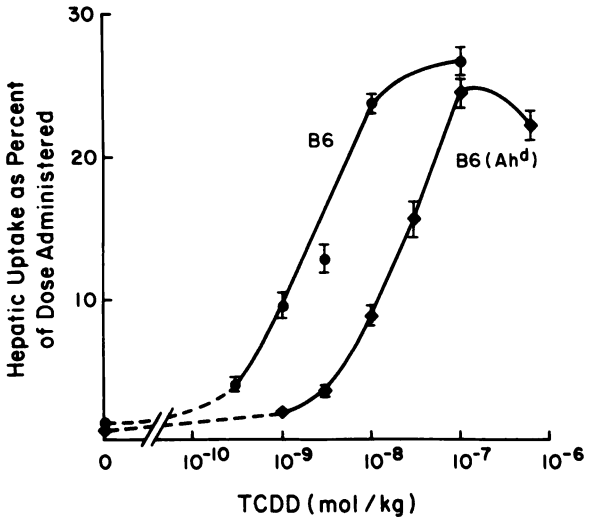

Fig. 5. Log dose-response curves for TCDD stimulation of hepatic uptake of [^{125}I]Cl_3D*p*D in C57BL/6J and congenic C57BL/6J (Ah^d/Ah^d) mice. C57BL/6J and congenic C57BL/6J (Ah^d/Ah^d) mice (6-8 weeks old, three males and two females in each treatment group) were treated with varying doses of TCDD, intraperitoneally, and 48 hr later all mice received [^{125}I]Cl_3D*p*D (1×10^{-10} mol/kg) intraperitoneally. Twenty-four hours after the dose of radiolabel, the animals were killed and the radioactivity in their livers was quantified and expressed as percentage of the administered dose. The value of the mean ± standard error (n = 5) was determined for each treatment group and plotted as the response (hepatic uptake of radioactivity) versus the log of the dose of TCDD. The value for the control (solvent-treated) mice is plotted on the *ordinate*.

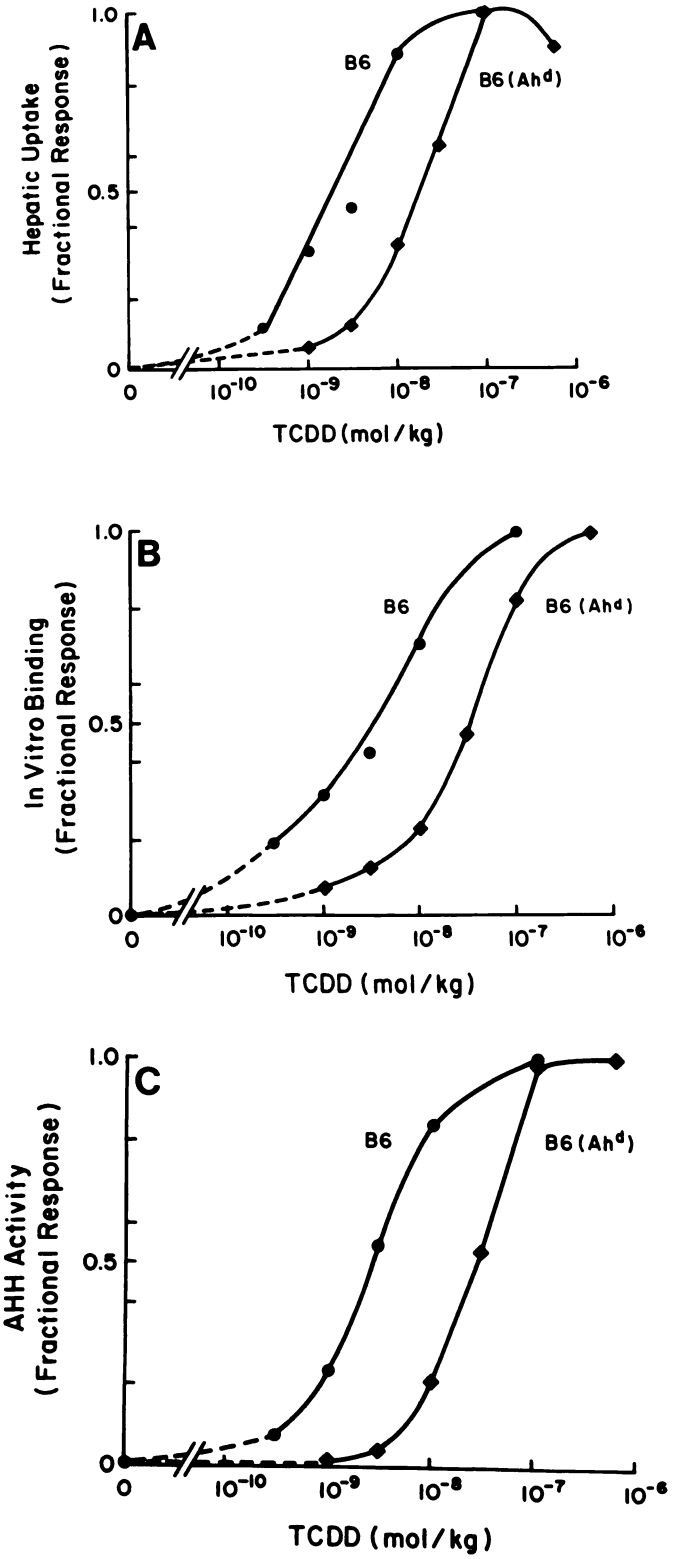

Fig. 6. Log-dose response curves for TCDD stimulation of hepatic uptake of [^{125}I]Cl_3DpD, *in vitro* binding of [^{125}I]Cl_3DpD, and induction of hepatic AHH activity in C57BL/6J and C57BL/6J (Ah^d/Ah^d) mice. A, hepatic uptake of [^{125}I]Cl_3DpD. The data from Fig. 7 are replotted as fractional response (control solvent-treated mice = 0; maximal response = 1.0). B and C, In a second experiment, C57BL/6J and C57BL/6J (Ah^d/Ah^d) 6–7-week-old mice of mixed sex (three female and two male per treatment group) were treated with varying doses of TCDD and killed 48 hr later. Liver homogenate binding of [^{125}I]Cl_3DpD and liver AHH activity were determined as described in Materials and Methods. For each treatment group, the mean value was determined and expressed as the fractional response. B, *In vitro* binding of [^{125}I]Cl_3DpD to liver homogenate. For C57BL/6J mice, the absolute value for total ligand binding in solvent-treated (control) mice was 5300 ± 400 dpm bound and for the maximally stimulated group 16,900 ± 800 dpm bound. Similar values were found for congenic C57BL/6J (Ah^d/Ah^d) mice. C, Hepatic AHH activity. For C57BL/6J mice, the absolute value for enzyme activity for control solvent-treated mice was 6.7 ± 1.1 units and for maximally induced mice 82.4 ± 4.2 units. Similar values were obtained for congenic C57BL/6J (Ah^d/Ah^d) mice.

Association of *in vivo* hepatic uptake and *in vitro* liver homogenate binding of [^{125}I]Cl_3DpD. Many of the biological effects produced by TCDD and related halogenated aromatic hydrocarbons, e.g., the induction of cytochrome P-450 and associated monooxygenase activity, are mediated by the stereospecific reversible binding of these compounds to the Ah receptor (2). C57BL/6J mice express a high affinity Ah receptor and are sensitive to induction of hepatic AHH activity (and other biological responses) by TCDD and less potent agonists, e.g., 3,3′,4,4′,5,5′-hexabromobiphenyl (2). Congenic C57BL/6J (Ah^d/Ah^d) mice express a low affinity receptor, are approximately 10-fold less sensitive to TCDD, and are unresponsive to weak agonists.

We examined the effect of TCDD administration on hepatic uptake of [^{125}I]Cl_3DpD, *in vitro* binding of this radioligand to liver homogenate, and hepatic AHH activity in C57BL/6J mice and congenic C57BL/6J (Ah^d/Ah^d) mice. As seen in Fig. 5, TCDD produced a dose-related increase in hepatic uptake of radioactivity of similar magnitude in both strains of mice, but the C57BL/6J (Ah^d/Ah^d) mice are approximately 10-fold less sensitive. In Fig. 6 are plotted the dose-response curves for all three parameters expressed as fractional responses. In C57BL/6J mice the dose of TCDD that produces one half the maximal response, ED_{50}, for each of these three responses was similar (ED_{50} values of 1.5 to 4.0 × 10^{-9} mol/kg), and in C57BL/6J (Ah^d/Ah^d) mice the ED_{50} values were 10-fold greater.

For the weak agonist 3,3′,4,4′,5,5′-hexabromobiphenyl, the dose-response curves for induction of hepatic uptake of [^{125}I]Cl_3DpD, *in vitro* binding of the ligand, and hepatic AHH activity are similar in C57BL/6J mice, with ED_{50} values of 5 to 6 × 10^{-7} mol/kg (Fig. 7). The maximal increase in all three parameters occurred at a dose of 3.5 × 10^{-6} mol/kg, whereas higher doses produced a decrease in response, possibly due to toxicity. This weak agonist did not produce any increase in these parameters in C57BL/6J (Ah^d/Ah^d) mice.

In Table 3 are shown the effects of administration of other compounds on these three parameters. TCDD, TCDBF, β-naphthoflavone, and benz(*a*)anthracene, agonists for the Ah receptor (6), increased all three responses, whereas congeners that do not bind to the receptor (6), e.g., 1,3,6,8-tetrachlorodibenzo-*p*-dioxin, 2,7-dichlorodibenzo-*p*-dioxin, and pyrene, produced no effect. We further examined a series of compounds that are not agonists for the Ah receptor and that induce cytochrome P-450 isozymes distinct from those of TCDD, barbiturate-like inducers [sodium phenobarbital, 2,2′,4,4′,5,5′-hexabromobiphenyl (13), and TCPOBOP (14)] and pregnenolone-16α-carbonitrile (15). These compounds did not appreciably increase hepatic uptake of [^{125}I]Cl_3DpD, although some did produce a moderate increase in AHH activity (presumably because this is an aggregate enzyme activity reflecting the contribution of several cytochrome P-450 isozymes).

The effect of TCDD administration on these responses in the rat, hamster, and guinea pig is shown in Table 4. TCDD

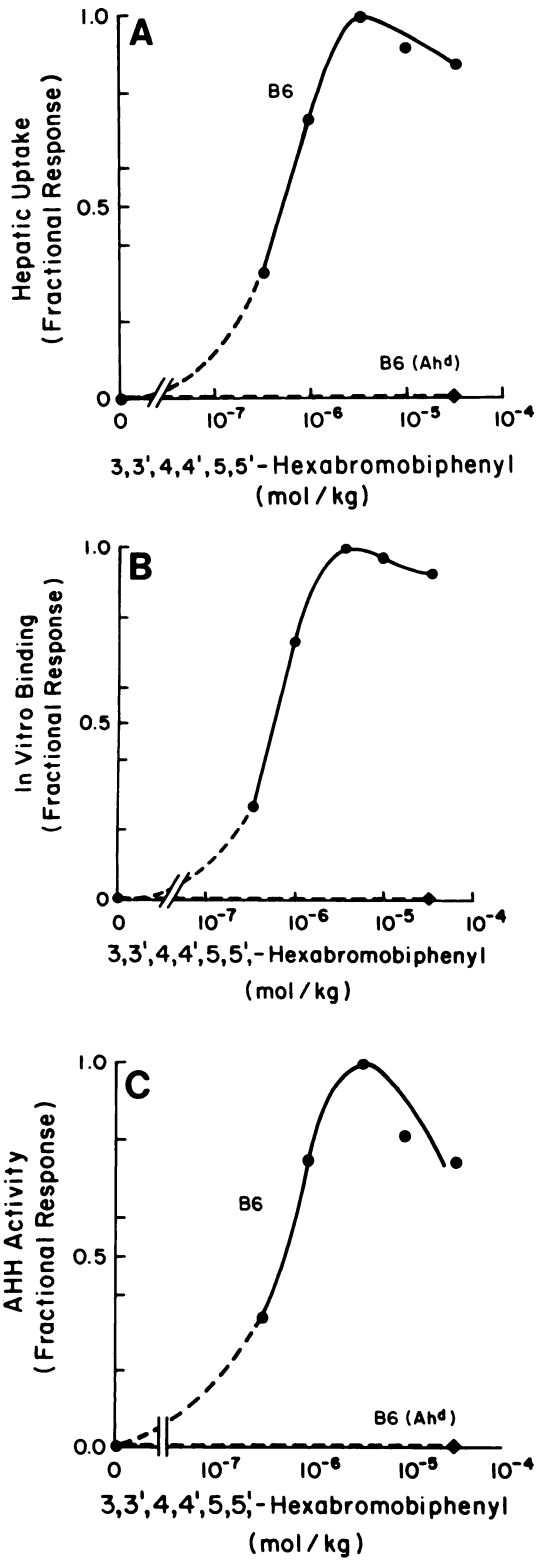

Fig. 7. Log-dose response curves for 3,3′,4,4′,5,5′-hexabromobiphenyl stimulation of hepatic uptake of [^{125}I]Cl_3DpD, *in vitro* binding of [^{125}I]Cl_3DpD, and hepatic AHH activity in C57BL/6J and C57BL/6J (*Ah*^{d}/*Ah*^{d}) mice. A, Hepatic uptake. C57BL/6J and C57BL/6J (*Ah*^{d}/*Ah*^{d}) female mice (7–8 weeks old) were treated with varying doses of 3,3′,4,4′,5,5′-hexabromobiphenyl in DMSO (4 ml/kg) intraperitoneally. Forty-eight hours later all mice were treated with [^{125}I]Cl_3DpD (1×10^{-10} mol/kg in *p*-dioxane, 0.3 ml/kg). Twenty-four hours after the administration of radiolabel, the mice were killed and hepatic radioactivity was quantified and expressed as fractional response. For C57BL/6J mice, the absolute values of hepatic uptake expressed as percentage of the dose administered were, for control (solvent-treated) mice, 2.22 ± 0.19% and maximally induced, 25.02 ± 0.60%. B and C, In a separate experiment, mice of the same ages and sex as in A were similarly treated with 3,3′,4,4′,5,5′-hexabromobiphenyl and killed 48 hr later. *In vitro* binding of [^{125}I]Cl_3DpD and hepatic AHH activity were determined. The values are expressed as fractional responses. B, *In vitro* binding of [^{125}I]Cl_3DpD. The absolute values for total *in vitro* binding in C57BL/6J mice were, for control, 4,100 ± 300 dpm and, for maximally induced mice, 11,300 ± 400. C, AHH activity. The absolute values for AHH activity in C57BL/6J mice were, for control, 9.72 ± 1.03 units and, maximally induced, 76.24 ± 4.10 units.

TABLE 3

Effect of administration of various compounds on hepatic uptake of radioligand *in vivo*, binding *in vitro*, and AHH activity in C57BL/6J mice

C57BL/6J female mice, 9–11 weeks old, were treated with the test compound intraperitoneally and 48 (hr) later (or, as indicated for mice treated with saline, phenobarbital, or pregnenolone-16-α-carbonitrile, 72 hr later) the animals were (a) killed for measurement of ligand binding and AHH activity *in vitro* or (b) treated with [^{125}I]Cl_3DpD intraperitoneally, with hepatic uptake measured 24 hr later. Each value is the mean ± standard error of determination on four or five animals.

Treatment	Hepatic Uptake	AHH Activity	In Vitro Binding
	% of dose administered	pmol/mg/min	dpm × 10^{-3}/0.25 mg of homogenate
Control (*p*-dioxane), 0.3 ml/kg	2.2 ± 0.1	7.3 ± 0.6	2.89 ± 0.15
TCDD, 3×10^{-8} mol/kg	30.3 ± 3.1	83.9 ± 3.0	12.14 ± 0.80
TCDBF, 3×10^{-7} mol/kg	25.5 ± 2.6	82.7 ± 3.1	12.24 ± 0.48
1,3,6,8-Tetrachlorodibenzo-*p*-dioxin, 3×10^{-7} mol/kg	2.4 ± 0.2	7.3 ± 0.9	3.94 ± 0.08
2,7-Dichlorodibenzo-*p*-dioxin, 3×10^{-7} mol/kg	2.2 ± 0.1	7.6 ± 0.5	3.79 ± 0.29
Control (DMSO), 4.0 ml/kg	2.4 ± 0.2	6.5 ± 0.3	3.48 ± 0.30
β-Naphthoflavone, 80 mg/kg/day, × 2	27.7 ± 2.1	86.8 ± 2.9	14.59 ± 0.68
Pyrene, 80 mg/kg/day × 2	2.0 ± 0.3	6.7 ± 0.7	4.34 ± 0.28
Benz(*a*)anthracene, 80 mg/kg	11.7 ± 0.6	35.5 ± 0.8	5.76 ± 0.10
Control (saline), 10 ml/kg/day × 3	1.7 ± 0.1	8.7 ± 0.4	3.52 ± 0.28
Sodium phenobarbital, 100 mg/kg/day, × 3	2.1 ± 0.1	31.7 ± 2.0	4.41 ± 0.51
Pregnenolone-16α-carbonitrile, 25 mg/kg/day × 3	2.2 ± 0.2	29.7 ± 0.8	4.43 ± 0.49
Control (DMSO), 4 ml/kg, 1×	1.9 ± 0.1	7.7 ± 0.4	4.02 ± 0.09
2,2′,4,4′,5,5′-Hexabromobiphenyl, 50 mg/kg, 1×	2.5 ± 0.3	18.2 ± 4.1	5.80 ± 0.58
3,3′,4,4′,5,5′-Hexabromobiphenyl, 20 mg/kg, 1×	27.0 ± 0.7	60.6 ± 3.4	9.64 ± 0.17
TCPOBOP, 3 mg/kg, 1×	2.9 ± 0.2	34.9 ± 2.0	6.67 ± 0.20

stimulated all three responses in these species. *In vivo* hepatic uptake of [^{125}I]Cl_3DpD is a more reliable measure of stimulation than *in vitro* binding, because the latter was not corrected for nonspecific binding and was not optimized for each species.

Discussion

TCDD administration to C57BL/6J mice produces a dose-related increase in the hepatic uptake of [^{125}I]Cl_3DpD *in vivo* and in the *in vitro* binding of this radioligand to liver homogenate from these mice. Several lines of evidence indicate these effects are mediated through the Ah receptor. (a) The ED_{50} values for TCDD stimulation of these responses and for the induction of hepatic AHH activity are similar (1.5 to 4.0×10^{-9} mol/kg) in C57BL/6J mice and are approximately 10-fold higher in congenic C57BL/6J (*Ah*^{d}/*Ah*^{d}) mice. (b) The weak agonist 3,3′,4,4′,5,5′-hexabromobiphenyl stimulates all three responses in C57BL/6J mice but not C57BL/6J (*Ah*^{d}/*Ah*^{d})

TABLE 4

Effect of TCDD on *in vivo* hepatic uptake and *in vitro* binding of radioligand and AHH activity in rat, hamster, and guinea pig

Sprague-Dawley rats (female, 100–115 g) and golden syrian hamsters (female, 60–65 g) were treated with *p*-dioxane (0.3 ml/kg) or TCDD (1×10^{-7} mol/kg) in the same vehicle, intraperitoneally. Forty-eight hours later, one group of animals were treated with ^{125}I radioligand (1×10^{-10} mol/kg) and killed 24 hr later to determine hepatic uptake; a second group of animals were killed and hepatic AHH activity and *in vitro* binding of radioligand to liver homogenate was determined. Guinea pigs (male, 215–260 g) were similarly treated with the following differences: the dose of TCDD was 3×10^{-8} mol/kg and the same animals were used for hepatic uptake *in vivo* and the *in vitro* studies, i.e., 48 hr after initial administration they received the radioligand and were killed 24-hr later. Thus, hepatic AHH activity and ligand binding were determined 72 hr after initial dosing.

	n	Hepatic Uptake	Hepatic AHH Activity	*In Vitro* Binding
		% of total dose	*pmol/mg/min*	*dpm* $\times 10^{-3}$*/0.25 mg of homogenate*
Rat				
Control	3	6.7 ± 1.5	1.6 ± 0.2	2.58 ± 0.14
TCDD	3	46.5 ± 1.6 (6.9)*	32.4 ± 2.1 (20.3)	4.77 ± 0.09 (1.8)
Hamster				
Control	3	7.2 ± 1.2	7.9 ± 1.9	2.14 ± 0.17
TCDD	3	30.8 ± 3.6 (4.3)	16.8 ± 0.5 (2.1)	9.93 ± 0.29 (4.6)
Guinea pig				
Control	3	2.0 ± 0.4	5.3 ± 0.4	3.74 ± 0.47
TCDD	3	6.5 ± 1.6 (3.3)	15.9 ± 1.1 (3.0)	5.28 ± 0.83 (1.4)

* Ratio of values for TCDD-treated/control.

mice. (c) The structure-activity relationship for stimulation of these responses corresponds to that for Ah receptor binding. Compounds that induce other cytochrome P-450 isozymes, e.g., barbiturate-like inducers and pregnenolone-16α-carbonitrile, do not stimulate hepatic uptake of [^{125}I]Cl_3DpD. TCDD administration induces these responses in the four laboratory species examined.

Because stimulation of hepatic uptake of [^{125}I]Cl_3DpD or [^{3}H]TCDD (8) *in vivo* is highly correlated with stimulation of binding of these radioligands to liver homogenate *in vitro*, the simplest, most parsimonious explanation is provided by the assumption these two are related, both manifestations of the same response. We propose that TCDD and other agonists act on the Ah receptor to "enhance" a binding moiety in the liver and this enhanced species is responsible for increased hepatic uptake *in vivo* and increased binding *in vitro*. The data presented in this report do not permit us to comment on the mechanisms of this stimulation (e.g., *de novo* protein synthesis, decreased degradation, or activation of this binding species). The accompanying report (12) provides a characterization of this binding species.

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