Biochemical Pharmacology, Vol. 33, No. 4, pp. 531–538, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

ELEVATED BINDING OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN AND 3-METHYLCHOLANTHRENE TO THE *Ah* RECEPTOR IN HEPATIC CYTOSOLS FROM PHENOBARBITAL-TREATED RATS AND MICE*

ALLAN B. OKEY† and LYNN M. VELLA

Division of Clinical Pharmacology, Department of Paediatrics, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8

(*Received* 19 *January* 1983; *accepted* 15 *July* 1983)

Abstract—Binding of 3-methylcholanthrene (MC), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and other "MC-type" inducers to cytosolic *Ah* receptor sites is the first specific step in induction of aryl hydrocarbon hydroxylase (AHH; cytochrome P_1-450) by these compounds. [^{3}H]TCDD and [^{3}H]MC were used as radioligands to quantitate and characterize *Ah* receptor in hepatic cytosols from genetically "responsive" C57BL/6J mice, genetically "nonresponsive" DBA/2J mice, and AHH-inducible Sprague–Dawley rats. Injection of 50–100 mg/kg of phenobarbital (PB) for 3 days more than doubled the concentration of *Ah* receptor in hepatic cytosol from Sprague–Dawley rats. In C57BL/6J mice, PB injection at 25 mg/kg × 3 days significantly increased ($P < 0.01$) the *Ah* receptor concentration in hepatic cytosol. No cytosolic *Ah* receptor was detectable in hepatic cytosol from untreated DBA/2J mice, nor did any *Ah* receptor appear after PB treatment in this "nonresponsive" strain. Although PB significantly elevated *Ah* receptor in hepatic cytosols of responsive rodents, many previous studies have shown that the maximal level of AHH activity in animals given PB and an "MC-type" inducer simultaneously is additive rather than synergistic. *Ah* receptor concentrations can be doubled by PB treatment without doubling the subsequent AHH-induction response to "MC-type" compounds. Thus, the cytosolic *Ah* receptor concentration *per se* may not be the primary determinant of a given tissue's maximal capacity for AHH induction by "MC-type" compounds.

The *Ah* receptor is a major regulatory gene product of the *Ah* gene complex which regulates induction of aryl hydrocarbon hydroxylase (AHH‡; cytochrome P_1-450) and several associated drug-metabolizing enzyme activities in liver and in non-hepatic tissues [1, 2]. Many polycyclic aromatic compounds (3-methylcholanthrene, benzo[*a*]pyrene, chlorinated dibenzo-*p*-dioxins, polychlorinated biphenyls, etc.) bind to the cytoplasmic *Ah* receptor as the first step in P_1-450 induction [1], after which the inducer·receptor complex translocates from the cytoplasm into the nucleus via a temperature-dependent process [3].

Although the receptor regulates P_1-450 induction, little is known about factors which control levels of the receptor itself. Since PB stimulates a broad spectrum of responses in liver, the effect of PB injection on *Ah* receptor levels was examined.

Animals used in the study were C57BL/6J mice, DBA/2J mice, and Sprague–Dawley rats. C57BL/6 mice are classified as genetically "responsive" at the *Ah* locus. That is, they exhibit AHH induction when treated with MC. DBA/2 mice have been classified as "nonresponsive" at the *Ah* locus since they do not exhibit significant AHH induction when treated with MC. DBA/2 mice do exhibit AHH induction when treated with TCDD, but DBA/2 mice require a TCDD dose approximately 15-fold higher than that required in C57BL/6 mice to achieve the same level of AHH induction [4]. The nonresponsiveness to MC and the reduced sensitivity to TCDD in DBA/2 mice appear to be be due to a defect in the *Ah* receptor. *Ah* receptor in DBA/2 mice does not bind [^{3}H]TCDD with sufficient affinity to allow detection of receptor in cytosols assayed *in vitro*. The *Ah* receptor is not totally absent in DBA/2 mice, however, as evidenced by recovery of a [^{3}H]TCDD·receptor complex from hepatic nuclei of DBA/2 mice injected *in vivo* with a large dose of [^{3}H]TCDD [5, 6]. Thus, the terms "responsive" and "nonresponsive" refer to relative sensitivity to AHH induction rather than absolute presence or absence of a response [4].

MATERIALS AND METHODS

Chemicals. [^{3}H]TCDD (ring-labeled, 50 Ci/mmole, 80% chemical purity) and nonradioactive TCDD were purchased from KOR Isotopes (Cambridge, MA); [^{3}H]MC (generally-labeled, 37 Ci/mmole, 95% chemical purity) was from the Amersham Corp. (Oakville, Ontario); nonradioactive MC

* A preliminary report of this work was presented at the Fifth International Symposium on Microsomes and Drug Oxidation, Tokyo, Japan, July 1981.

† Supported by a grant from the Medical Research Council of Canada. Author to whom all correspondence should be addressed.

‡ Abbreviations: AHH, aryl hydrocarbon hydroxylase; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; MC, 3-methylcholanthrene; PB, phenobarbital; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

from Eastman Organic Chemicals (Rochester, NY); and HEPES from the Calbiochem-Behring Corp. (La Jolla, CA). Dextran and dithiothreitol were from the Sigma Chemical Co. (St. Louis, MO); sucrose (SDG grade) was from Beckman Instruments (Toronto, Ontario); and dimethyl sulfoxide, glycerol, charcoal (Norit A) and ethylenediaminetetraacetic acid were from the Fisher Scientific Co. (Toronto, Ontario). Phenobarbital was supplied through the courtesy of Mr. R. A. Graham, Health Protection Branch, Health and Welfare Canada.

Animals. Male Sprague–Dawley rats were purchased from Camm Research Lab. Animals (Wayne, NJ); cytosol was prepared from animals weighing between 200 and 250 g. Male C57BL/6J and DBA/2J mice were purchased from The Jackson Laboratory (Bar Harbor, ME); cytosol was prepared from these animals when they were between the ages of 3 and 6 months. Genetically "responsive" heterozygous Ah^b/Ah^d mice and homozygous "nonresponsive" Ah^d/Ah^d mice were produced in our laboratory by backcrossing F_1 offspring from the C57BL/6J × DBA/2J cross with "nonresponsive" DBA/2J mice. Offspring from the backcross were phenotyped for responsiveness at the *Ah* locus by measurement of their zoxazolamine paralysis time [7]. All animals were housed in wire cages, exposed to 12 hr light: 12 hr dark each day, and were permitted free access to water and food (Purina Laboratory Chow) until the time of death. PB was injected intraperitoneally for 3 consecutive days before the animals were killed on day 4 for removal of the liver. Control animals were injected with 0.9% NaCl. Phenobarbital doses above 50 mg/kg caused sedation in both mice and rats, but no overt toxicity was evident in either species.

Buffer. The standard buffer used for all procedures was HEDG [25 mM HEPES; 1.5 mM ethylenediaminetetraacetic acid; 1 mM dithiothreitol; 10% (v/v) glycerol, pH 7.6].

Preparation of cytosol. Rats and mice were killed by cervical fracture. The livers were perfused *in situ* with iced HEDG buffer via a needle inserted into the inferior vena cava. The liver then was removed, rinsed with HEDG buffer, minced, and homogenized in HEDG buffer (5 ml/g liver) using a Polytron PT-10 (Brinkmann Instruments, Rexdale, Ontario). The homogenates were centrifuged at 4,000 *g* for 20 min, and the resulting supernatant fraction was centrifuged at 105,000 *g* for 1 hr. Cytosol was carefully drawn off with a Pasteur pipette without disturbing the surface lipid layer or the microsomal pellet. Protein concentrations were determined by the method of Bradford [8] using the

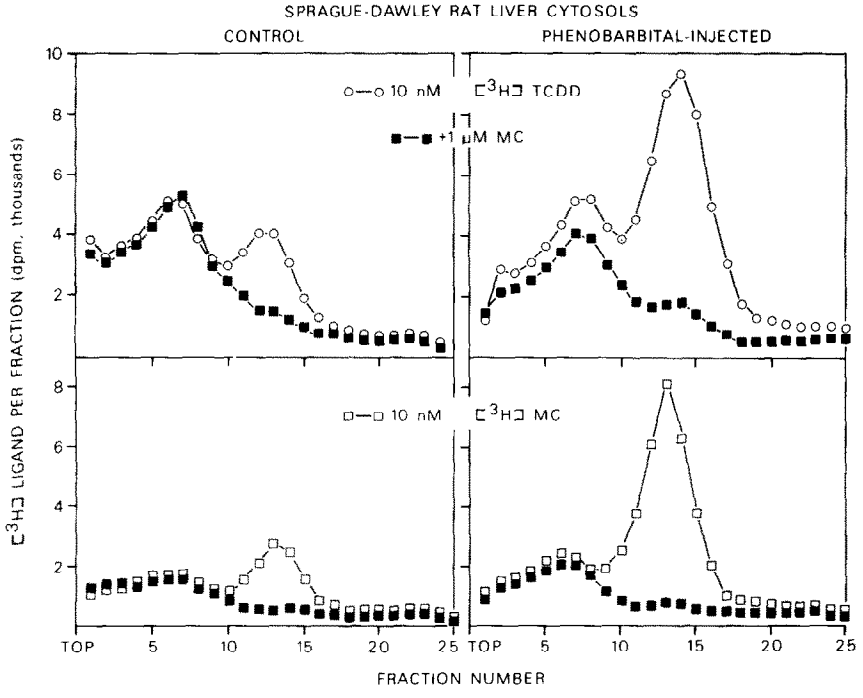

Fig. 1. Sucrose density gradient profiles of radioligand binding in hepatic cytosol from control versus phenobarbital-injected rats. Hepatic cytosols (5 mg protein/ml) were prepared from a control rat (injected with 0.9% NaCl) and a rat injected with PB (50 mg/kg body weight for 3 consecutive days before removal of the liver). Cytosols were incubated with 10 nM [^{3}H]TCDD (50 Ci/mmole) or with 10 nM [^{3}H]MC (37 Ci/mmole) in the absence (○—○) or presence (■—■) of a 100-fold molar excess of nonradioactive MC. Samples were treated with charcoal–dextran before analysis as described in Materials and Methods. Specific *Ah* receptor binding is represented by the peaks sedimenting at "9S" (near fraction 13 in these assays). The concentration of specific binding sites in the "9S" peak was calculated by subtracting binding in the presence of 1 μM MC from binding in the absence of competitor. In these particular assays, the concentrations of specific binding (fmoles/mg cytosol protein) were: [^{3}H]TCDD, control = 62, PB-injected = 149; [^{3}H]MC, control = 67, PB-injected = 161.

BioRad protein assay kit (BioRad Laboratories, Mississauga, Ontario). All procedures in the preparation and analysis of cytosol samples were performed at 0–4°.

Cytosol incubation. Samples for sucrose density gradient analysis were prepared by incubating 1 ml of cytosol (about 5 mg protein/ml) with 10 nM [^{3}H]TCDD or 10 nM [^{3}H]MC for 1 hr at 0–4° [1]. The radioligands were added to cytosol in 10 μl of dimethyl sulfoxide/ml of cytosol; dimethyl sulfoxide also was used as the solvent for nonradioactive TCDD and MC in competition experiments. Water was used as a solvent for PB in experiments testing the *in vitro* effect of PB on binding of [^{3}H]TCDD and [^{3}H]MC to the *Ah* receptor. In competition experiments, an amount of solvent equal to that used to introduce the competitor was added to the control (no competitor) sample. After incubation, unbound and loosely bound radioligands were removed by adding cytosol samples to a charcoal–dextran pellet (10 mg charcoal:1 mg dextran, pelleted from HEDG buffer). Charcoal–dextran was resuspended in the cytosol on a Vortex mixer, incubated with the sample for 15 min, and then removed by centrifugation at 4,000 *g* for 15 min.

Sucrose density gradient analysis. Aliquots (300 μl) of cytosol samples were layered onto linear sucrose gradients prepared in HEDG buffer. Gradients were centrifuged either for 16 hr at 48,000 rpm in a swinging-bucket rotor (Beckman SW 60Ti, g_{av} = 235,000) or for 2 hr in a vertical-tube rotor (Beckman VTi65, g_{av} = 372,000). The concentration of *Ah* receptor sites detected with the 2-hr separation is equal to that detected with a 16-hr separation [9]. After centrifugation, twenty 200-μl fractions were collected from each SW 60Ti gradient, or twenty-five 200-μl fractions were collected from each VTi65 gradient using an ISCO model 640 gradient fractionator (Instrumentation Specialties Co., Lincoln, NE). Radioactivity in each fraction was determined by liquid scintillation counting and corrected for counting efficiency.

RESULTS

Effect of phenobarbital injection on Ah *receptor concentrations in Sprague–Dawley rat liver.* Sucrose density gradient profiles in Fig. 1 illustrate the substantial increase in *Ah* receptor content which followed PB injection in the rat. Binding of both [^{3}H]TCDD and [^{3}H]MC in the 9S region of the gradient was increased by pretreatment of the rat with PB.

The specificity and limited capacity of the 9S component were confirmed by competition studies. As has been demonstrated previously [10], a 100-fold molar excess of nonradioactive MC in the incubation system eliminated binding of both [^{3}H]TCDD and [^{3}H]MC in the 9S region when assays were done on cytosols from control rats (Fig. 1, left-hand panels). The same concentration of MC also eliminated binding of both radioligands in the 9S region in assays on cytosols from PB-treated rats (Fig. 1, right-hand panels).

Specificity of the 9S component in cytosol from PB-treated rats is further illustrated in Fig. 2. A

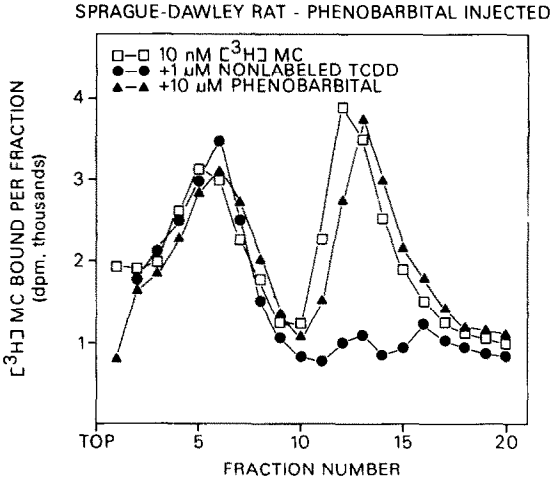

Fig. 2. Specificity of [^{3}H]MC binding in hepatic cytosol from a phenobarbital-treated rat. Hepatic cytosol (4.8 mg protein/ml) from a PB-injected rat (50 mg/kg × 3 days) was incubated *in vitro* with 10 nM [^{3}H]MC in the absence of potential competitors (□—□) or in the presence of a 100-fold molar excess of nonradioactive TCDD (●—●) or a 1000-fold molar excess of PB (▲—▲). After treatment with charcoal–dextran, samples were analyzed by velocity sedimentation on sucrose density gradients as described in Materials and Methods.

100-fold molar excess of nonradioactive TCDD eliminated binding of [^{3}H]MC in the 9S region, whereas a 1000-fold excess of PB in the *in vitro* incubation had no effect on binding of [^{3}H]MC (Fig. 2) nor [^{3}H]TCDD (data not shown). In cytosol from PB-treated rats, binding of [^{3}H]MC in the 9S region also was eliminated by 100-fold molar excesses of benz[*a*]anthracene and dibenz[*a*,*h*]anthracene (gradient profiles not shown). Overall, the 9S component from PB-treated rats exhibited the same specificity as the 9S component (*Ah* receptor) from control rats.

The concentration of *Ah* receptor in rat hepatic cytosols increased sharply at PB doses between 15 and 50 mg/kg (Fig. 3). *Ah* receptor content was doubled at a dose of 50 mg/kg, but no further increase in receptor concentration was achieved by doses higher than 50 mg/kg.

The concentration of *Ah* receptor is conventionally expressed as fmoles/mg cytosol protein as illustrated in Fig. 3. The dose–response curves were similar whether receptor was expressed as fmoles/g wet weight of liver or as fmoles/mg cytosol protein. The correlation between fmoles/mg cytosol protein and fmoles/g liver for [^{3}H]TCDD binding was: $r = 0.84$ ($N = 21$, $P < 0.001$). The elevation in *Ah* receptor content at different PB doses was moderately correlated with the extent of induction of microsomal protein in livers from the same animals ($r = 0.44$, $N = 21$, $P < 0.05$).

Effect of phenobarbital injection on [^{3}H]*TCDD and* [^{3}H]*MC binding in genetically "responsive" versus genetically "nonresponsive" mice.* In genetically "responsive" C57BL/6J mice not treated with PB, specific *Ah* receptor binding was detectable in the 9S region either with [^{3}H]TCDD (Fig. 4). or with

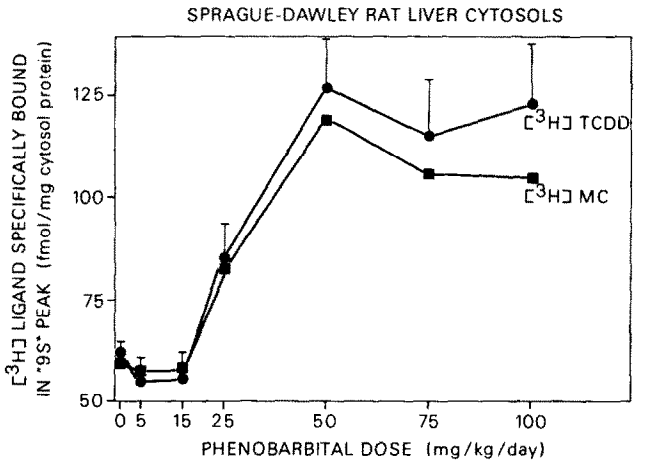

Fig. 3. Dose-dependent increase in hepatic *Ah* receptor in phenobarbital-treated rats. PB was injected I.P. once per day for 3 consecutive days at doses indicated in the figure. Livers were removed on day 4, and cytosols were prepared and analyzed for *Ah* receptor content using both [^{3}H]TCDD and [^{3}H]MC as described in Materials and Methods. Each point represents the mean ± S.E. for a group of three rats. Standard errors with [^{3}H]MC as the radioligand are not plotted, but were similar to those for [^{3}H]TCDD. When measured with either [^{3}H]TCDD or [^{3}H]MC, *Ah* receptor concentrations in rats receiving 25, 50, 75, or 100 mg/kg were significantly greater than control values ($P < 0.05$, by *t*-test).

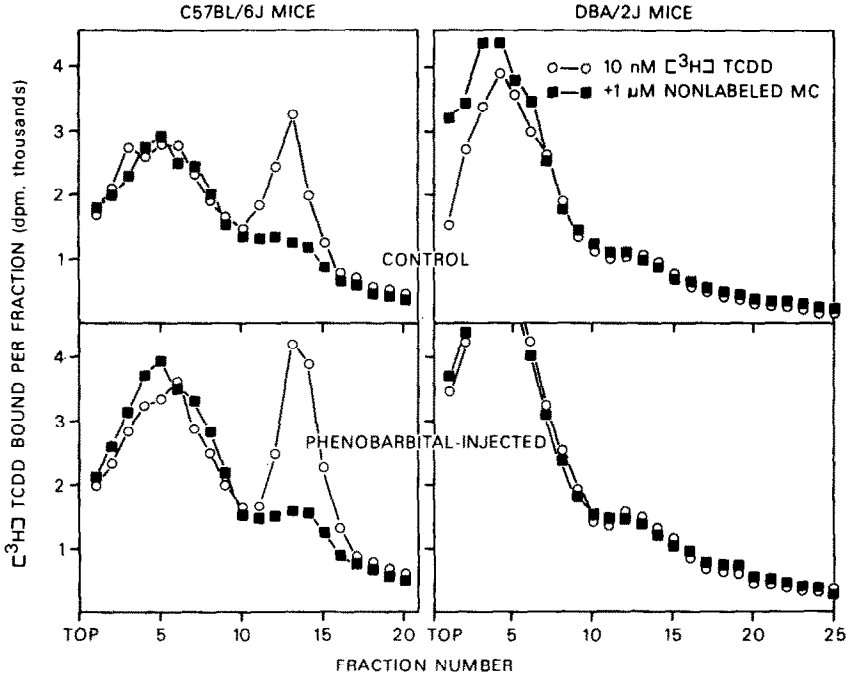

Fig. 4. Binding of [^{3}H]TCDD in genetically "responsive" C57BL/6J mice and in genetically "nonresponsive" DBA/2J mice. Hepatic cytosols (5 mg protein/ml) were prepared from control mice and from mice injected with PB (25 mg/kg for 3 consecutive days before removal of the livers) and then were incubated with 10 nM [^{3}H]TCDD in the absence (○—○) or presence (■—■) of a 100-fold molar excess of nonradioactive MC. Samples were treated with charcoal–dextran before separation on sucrose density gradients as described in Materials and Methods. In this assay, the concentration of specific *Ah* receptor (fmoles/mg cytosol protein) was 32 in the control C57BL/6J mouse and 43 in the PB-treated C57BL/6J mouse. Gradient profiles with 1 μM nonradioactive TCDD as the competitor were the same as with 1 μM nonradioactive MC (profiles not illustrated for TCDD as the competitor).

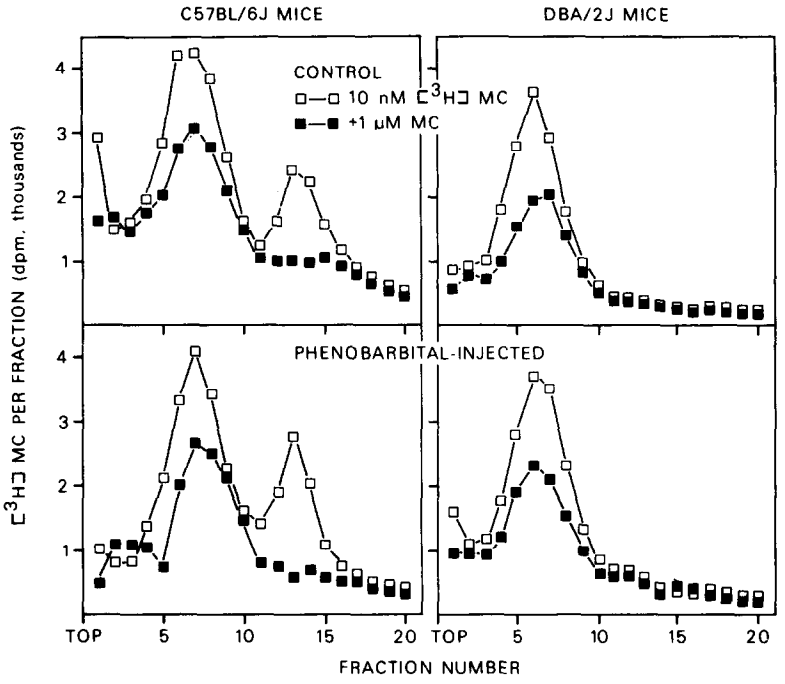

Fig. 5. Binding of [^{3}H]MC in genetically "responsive" C57BL/6J mice and in genetically "nonresponsive" DBA/2J mice. Hepatic cytosol samples from the same mice described in the legend to Fig. 4 were analyzed using [^{3}H]MC as the radioligand. The concentration of specific *Ah* receptor (fmoles/mg protein) detected in these samples with [^{3}H]MC as the radioligand was 36 in the control C57BL/6J mouse and 47 in the PB-treated mouse. Nonradioactive TCDD (1 μM) completely extinguished binding of [^{3}H]MC in the "9S" region (near fraction 13), but had no effect on [^{3}H]MC binding in the 4–5S region (near fraction 5) (data not illustrated).

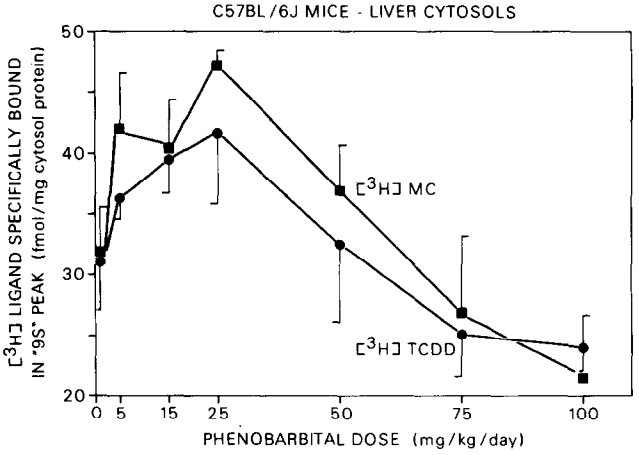

Fig. 6. Dose-related effects of phenobarbital on hepatic *Ah* receptor in C57BL/6J mice. PB was injected i.p. once per day for 3 consecutive days at doses indicated in the figure. Livers were removed on day 4 and cytosols were prepared and analyzed for *Ah* receptor content using both [^{3}H]TCDD and [^{3}H]MC as described in Materials and Methods. Each point represents the mean ± S.E. for a group of three mice. By *t*-test, none of the PB-treated groups had *Ah* receptor concentrations significantly different from the control group. However, the *Ah* receptor concentration with [^{3}H]MC as the radioligand was significantly greater ($P < 0.05$) in mice treated with 5, 15 or 25 mg/kg PB than in mice treated with 100 mg/kg.

Table 1. Effect of 25 mg phenobarbital/kg body weight on *Ah* receptor levels in hepatic cytosols from C57BL/6J mice*

Radioligand used in assay	*Ah* receptor content (fmoles/mg protein)	
	Control	PB-treated (25 mg/kg × 3 days)
[^{3}H]TCDD	29.6 ± 1.9	39.7 ± 2.7†
[^{3}H]MC	36.7 ± 1.6	49.2 ± 1.8†

* Mice were injected with 25 mg phenobarbital/kg body weight. Values are mean ± S.E., N = 7 for all groups.

† Significantly different from control ($P < 0.01$ by Student's *t*-test).

Table 2. Dissociation constants for [^{3}H]TCDD binding in hepatic cytosols from control versus phenobarbital-treated rats*

	K_d (nM)
Control	2.21 ± 0.36
Phenobarbital-treated (50 mg/kg × 3 days)	2.28 ± 0.14

* Specific *Ah* receptor binding of [^{3}H]TCDD was determined by sucrose density gradient assays at each of twelve different [^{3}H]TCDD concentrations over a range from 0.05 to 20 nM. Dissociation constants were derived from Scatchard plot analyses as described in the legend to Fig. 7. Values are expressed as mean ± S.E.M. (N = 4).

[^{3}H]MC (Fig. 5) as the radioligand. No specific cytosolic receptor was detectable in untreated "nonresponsive" DBA/2J mice using either radioligand (Figs. 4 and 5).

C57BL/6J mice treated with PB doses between 5 and 25 mg/kg had a slight increase in hepatic *Ah* receptor concentration as measured with either [^{3}H]TCDD or [^{3}H]MC (Fig. 6). With the small number of animals able to be tested at each dose, however, none of the increases was statistically significant. Therefore, a larger group of C57BL/6J mice was tested at the 25 mg/kg dose only. As shown in Table 1, the *Ah* receptor concentration in hepatic cytosol from C57BL/6J mice treated with 25 mg/kg PB was significantly elevated over the control level when assayed with either [^{3}H]TCDD or [^{3}H]MC.

At doses of 75 and 100 mg/kg, *Ah* receptor concentrations in C57BL/6J hepatic cytosols were lower than control values, but the differences were not statistically significant ($P > 0.05$).

In genetically "nonresponsive" DBA/2J mice, PB treatment did not lead to formation of any detectable cytosolic *Ah* receptor (Figs. 4 and 5). The possibility that PB treatment might stimulate production of *Ah* receptor in genetically "nonresponsive" animals also was tested in Ah^d/Ah^d "nonresponsive" backcross mice. As was the case with DBA/2J mice, treatment of "nonresponsive" Ah^d/Ah^d backcross mice with PB did not result in formation of any detectable cytosolic *Ah* receptor (data not shown).

In genetically "responsive" heterozygous Ah^b/Ah^d backcross mice, PB treatment produced a slight, but statistically nonsignificant, increase in *Ah* receptor content: 17.9 ± 3.6 fmoles/mg in control Ah^b/Ah^d

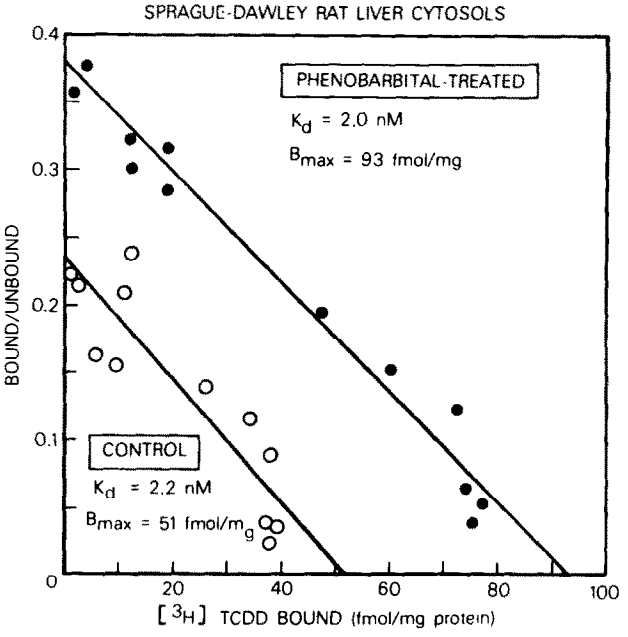

Fig. 7. Representative Scatchard plots comparing binding of [^{3}H]TCDD in hepatic cytosol from control versus phenobarbital-treated rats. Aliquots of hepatic cytosol from a control rat and from a phenobarbital-treated rat (50 mg/kg × 3 days) were incubated with twelve different concentrations of [^{3}H]TCDD over a range from 0.05 to 20 nM. Specific binding at each concentration was determined by sucrose density gradient analysis. "Unbound" [^{3}H]TCDD was computed by subtracting "specifically-bound" [^{3}H]TCDD from the total [^{3}H]TCDD present in the incubation. Binding parameters shown in the graphs were calculated by least squares linear regression.

mice compared with 23.3 ± 2.9 fmoles/mg in Ah^b/Ah^d mice treated with 25 mg/kg PB (N = 4 for each group; $P > 0.05$).

Affinity of [^{3}H]*TCDD binding after phenobarbital treatment.* The affinity with which [^{3}H]TCDD bound to *Ah* receptor in rat hepatic cytosol was unaltered by PB treatment (Table 2). Scatchard plots shown in Fig. 7 illustrate that the effect of PB treatment was to increase the concentration of *Ah* receptor sites without any alteration in the affinity with which the elevated receptor population bound [^{3}H]TCDD.

DISCUSSION

Binding of both [^{3}H]TCDD and [^{3}H]MC was elevated significantly in hepatic cytosol from genetically "responsive" C57BL/6J mice injected with 25 mg/kg PB. The elevation in binding was even more pronounced in hepatic cytosol from rats injected with 50–100 mg/kg.

Properties of the PB-induced binding component are consistent with properties previously described for the *Ah* receptor [1, 3, 10–13]. It sediments near 9S on gradients of low ionic strength when labeled with either [^{3}H]TCDD or [^{3}H]MC, and the binding of each radioligand is preferentially inhibited by TCDD, MC, dibenz[*a*,*h*]anthracene and benz[*a*]anthracene, compounds that are potent inducers of cytochrome P_1-450. Presence of the PB-induced 9S binding component also segregated with the *Ah* genetic locus. No specific 9S binding of either [^{3}H]TCDD or [^{3}H]MC could be detected in cytosol from untreated genetically "nonresponsive" DBA/2J or "nonresponsive" Ah^d/Ah^d backcross mice, nor did any 9S component appear in "nonresponsive" mice after PB injection.

In vitro addition of PB to cytosol while it was being incubated with [^{3}H]TCDD or [^{3}H]MC did not alter binding of the radioligands to the 9S component. Thus, the elevation in binding of [^{3}H]TCDD and [^{3}H]MC to cytosols from PB-treated animals appears to be due to increased levels of *Ah* receptor in the cytosols and is not caused by PB potentiating the *in vitro* interaction between the receptor and the radioligands. It also is possible, but less likely, that PB increases available *Ah* receptor sites by decreasing some endogenous component that was restricting binding of the radioligands.

Scatchard plots of [^{3}H]TCDD binding in hepatic cytosols from PB-treated rats were linear and the slopes were the same as Scatchard plots derived from analyses on cytosol from control rats. This indicates that, although the total *Ah* receptor concentration was increased by PB treatment, the elevated portion (induced by PB) had the same affinity as the constitutive *Ah* receptor which was present before PB treatment.

These experiments demonstrate that PB can increase the concentration of *Ah* receptor sites in hepatic cytosols from "responsive" rodents. "MC-type" enzyme inducers do not appear to stimulate elevation of *Ah* receptor levels. Injection of β-naphthoflavone causes a depletion of hepatic cytosolic *Ah* receptor sites in C57BL/6N mice [1], and incubation of hepatoma cells in culture with benz[*a*]anthracene does not increase either cytosolic or nuclear *Ah* receptor concentrations [3]. These assays measured only unoccupied *Ah* receptor sites, however. If the "MC-type" compounds administered not only elevated *Ah* receptor levels, but also occupied those sites, the sites would not have been detected with the techniques used in the previous studies. An exchange assay which potentially can measure both occupied and unoccupied *Ah* receptor sites has been reported [12] but has not yet been applied to determine whether "MC-type" compounds induce their own receptor.

Although PB treatment elevated *Ah* receptor levels in hepatic cytosol from "responsive" rodents, PB does not synergize with "MC-type" compounds to cause superinduction of enzyme activities associated with cytochrome P_1-450. Many studies have shown that *simultaneous* treatment with a "PB-like" inducer and an "MC-type" inducer may, at best, produce AHH activities that are additive, but not synergistic [14–19].

Fewer studies have tested the effect of *pretreatment* with PB on the subsequent response to MC. Polyakova *et al.* [20] found that pretreatment of genetically "nonresponsive" DBA/2 mice with PB did not convert them into a condition where they were "responsive" to later induction of AHH by MC. Harada and Omura [21], by immunologic techniques, demonstrated that PB and MC induce their own specific forms of cytochrome P-450 by independent mechanisms. In their experiments injection of MC into rats previously treated for 5 days with PB led to a rapid increase in the form of cytochrome P-450 induced by MC, but the level of $P\text{-}450_{MC}$ achieved was no greater in rats pretreated with PB than in rats given MC alone.

As shown in this report, the *Ah* receptor level in rat hepatic cytosol can be doubled by PB treatment, but this does not lead to a doubling of the response to subsequent treatment with "MC-type" enzyme inducers. In inbred mice and their F_1 progeny, the maximal level of induced AHH correlates with the concentration of cytosolic *Ah* receptor up to a concentration of about 1000 receptor sites per liver cell. Above 1000 receptor sites per cell, however, the increase in maximal AHH is less than the increase in receptor concentration [4].

Binding to the *Ah* receptor is essential for induction of cytochrome P_1-450 and associated enzymes regulated by the *Ah* gene complex [1, 2]. However, presence of cytosolic *Ah* receptor in a tissue or cell does not guarantee that that tissue or cell will exhibit enzyme induction when exposed to "MC-type" chemicals [3, 22–24]. Nor does the level of cytosolic *Ah* receptor seem to be the sole or primary determinant of a tissue's maximal capacity for induced AHH activity. Several pharmacologic response systems are known in which there are "spare receptors" [25]. The concept of "spare receptors" may apply also to cytosolic *Ah* receptor. Maximal response capacity for processes regulated via the *Ah* receptor may depend less on cytosolic receptor concentrations than on subsequent action of the inducer·receptor complex at the nuclear level [6].

Elevation of *Ah* receptor content is just one of many pleiotypic effects which PB has on rodent liver. Thus far, PB treatment is the only known procedure

by which *Ah* receptor concentrations can be increased over the constitutive basal level.

REFERENCES

1. A. B. Okey, G. P. Bondy, M. E. Mason, G. F. Kahl, H. J. Eisen, T. M. Guenthner and D. W. Nebert, *J. biol. Chem.* **254**, 11636 (1979).
2. D. W. Nebert, H. J. Eisen, M. Negishi, M. A. Lang, L. M. Hjelmeland and A. B. Okey, *A. Rev. Pharmac. Toxic.* **21**, 431 (1981).
3. A. B. Okey, G. P. Bondy, M. E. Mason, D. W. Nebert, C. J. Forster-Gibson, J. Muncan and M. J. Dufresne, *J. biol. Chem.* **255**, 11415 (1980).
4. H. J. Eisen, R. R. Hannah, C. Legraverend, A. B. Okey and D. W. Nebert, in *Biochemical Actions of Hormones* (Ed. G. Litwack), Vol. 10, p. 227. Academic Press, New York (1983).
5. M. E. Mason and A. B. Okey, *Eur. J. Biochem.* **123**, 209 (1982).
6. R. H. Tukey, R. R. Hannah, M. Negishi, D. W. Nebert and H. J. Eisen, *Cell* **31**, 275 (1982).
7. J. R. Robinson and D. W. Nebert, *Molec. Pharmac.* **10**, 484 (1974).
8. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
9. H. W. Tsui and A. B. Okey, *Can. J. Physiol. Pharmac.* **59**, 927 (1981).
10. A. B. Okey and L. M. Vella, *Eur. J. Biochem.* **127**, 39 (1982).
11. R. R. Hannah, D. W. Nebert and H. J. Eisen, *J. biol. Chem.* **256**, 4584 (1981).
12. L. Poellinger, R. N. Kurl, J. Lund, M. Gillner, J. Carlstedt-Duke, B. Hogberg and J-A. Gustafsson, *Biochim. biophys. Acta* **714**, 516 (1982).
13. A. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251**, 4936 (1976).
14. J. E. Gielen and D. W. Nebert, *J. biol. Chem.* **246**, 5189 (1971).
15. H. Vainio and A. Aitio, *Acta pharmac. tox.* **34**, 130 (1974).
16. J. G. Dent, K. J. Netter and J. E. Gibson, *Toxic. appl. Pharmac.* **38**, 237 (1976).
17. T. M. Guenthner and G. J. Mannering, *Biochem. Pharmac.* **26**, 567 (1977).
18. A. Parkinson, R. Cockerline and S. Safe, *Biochem. Pharmac.* **29**, 259 (1980).
19. L. W. Robertson, A. Parkinson and S. Safe, *Biochem. biophys. Res. Commun.* **92**, 175 (1980).
20. N. E. Polyakova, I. B. Tsyrlov and V. V. Lyakhovich, *Byull. eksp. Biol. Med.* **90**, 167 (1980) [Translation 0007-4888/80/9008-1063 (Plenum Publishing Corp., 1981)].
21. N. Harada and T. Omura, *J. Biochem.* **89**, 237 (1981).
22. G. F. Kahl, D. Friederici, S. W. Bigelow, A. B. Okey and D. W. Nebert, *Devl Pharmac. Ther.* **1**, 137 (1980).
23. C. Legraverend, R. R. Hannah, H. J. Eisen, I. S. Owens, D. W. Nebert and O. Hankinson, *J. biol. Chem.* **257**, 6402 (1982).
24. A. G. Miller, D. Israel and J. P. Whitlock, *J. biol. Chem.* **258**, 3523 (1983).
25. A. Goldstein, L. Aronow and S. Kalman, *Principles of Drug Action*, pp. 101–4. John Wiley, New York (1974).