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Genetic Expression of Aryl Hydrocarbon Hydroxylase by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Evidence for a Receptor Mutation in Genetically Non-responsive Mice

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SUMMARY

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Hepatic aryl hydrocarbon hydroxylase activity is induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in all inbred strains of mice tested. The genetically "nonresponsive" strains of mice which fail to respond to the usual aromatic hydrocarbons (e.g., 3-methylcholanthrene) are induced by TCDD; however, the dose required is greater than for the genetically "responsive" strains. The dose of TCDD that elicits half the maximal enzyme activity (ED_{50}) in responsive strains, C57BL/6J, BALB/cJ, and A/J, is approximately 1 nmole/kg, and in the nonresponsive strains, DBA/2J, AKR/J, and SJL/J, the ED_{50} is at least 10 nmoles/kg. In the above strains, TCDD at 3 nmoles/kg fails to induce hepatic aryl hydrocarbon hydroxylase activity in nonresponsive mice, but elicits 70% or more of the observed maximal enzyme activity in responsive strains. In 14 inbred strains of mice, the phenotypic characteristic of aromatic hydrocarbon responsiveness or nonresponsiveness can be detected equally well by a challenge with 3-methylcholanthrene (0.3 mmole/kg) or TCDD at 3 nmoles/kg. In all nonresponsive strains, a 10-fold higher dose of TCDD induces hepatic enzyme activity. The heterozygous offspring of C57BL/6J and DBA/2J parents are distinguishable from both parental strains by an intermediate sensitivity to TCDD. We conclude from these data that the genetically nonresponsive mice have the structural and regulatory genes necessary for the expression of aryl hydrocarbon hydroxylase. The most likely explanation of the defect in nonresponsive mice appears to be a mutation which results in an induction receptor site with a diminished affinity for inducing drugs, leading to an almost absolute unresponsiveness to 3-methylcholanthrene and a diminished sensitivity to the more potent inducer, TCDD.

INTRODUCTION

Aryl hydrocarbon hydroxylase is a cytochrome P-450-mediated microsomal monooxygenase that metabolizes numerous aromatic drugs, insecticides, and carcinogens. The importance of aromatic hydroxylation of xenobiotics to pharmacology, toxicology, and chemical carcinogenesis has been recently reviewed (1).

The administration of 3-methylcholanthrene or β-naphthoflavone to certain in-

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bred strains of mice induces[1] aryl hydrocarbon hydroxylase activity and three other microsomal monooxygenase activities (2–5), and results in the formation of a spectrally distinct cytochrome termed P_1-450, which is the presumed enzyme active site for these monooxygenases (6, 7). C57BL/6 is the prototype strain for these genetically responsive mice. In other inbred strains of mice, termed genetically nonresponsive, the administration of MC[2] does not produce these changes, or evokes only minimal changes. DBA/2 is the prototype nonresponsive strain. The induction of aryl hydrocarbon hydroxylase activity and the three other monooxygenase activities and the formation of cytochrome P_1-450 by aromatic hydrocarbons are all inherited in a simple autosomal dominant mode in genetic crosses and back-crosses between C57BL/6 and DBA/2 mice. These data suggested that these activities are all expressed at a single locus or at a group of closely linked loci (2, 3). Recently Robinson *et al.* (8), in an extensive survey of crosses and back-crosses among 12 strains of inbred mice, have reported evidence that the inheritance of aromatic hydrocarbon responsiveness is controlled by several nonlinked loci.

We have shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, an extremely toxic contaminant formed during the commercial synthesis of the herbicide 2,4,5-trichlorophenoxyacetic acid, is the most potent inducer of aryl hydrocarbon hydroxylase and cytochrome P_1-450 known; it is 30,000 times more potent than MC as an inducer of hepatic hydroxylase activity in the rat (9).

MC and TCDD induce hydroxylase activity in rat liver to the same maximal level, and simultaneous administration of maximally inducing doses of both drugs results in no additive effect; moreover, MC and TCDD produce log-dose response curves having parallel slopes. These observations suggest that the two drugs share a common mechanism of induction of hydroxylase activity.

In both genetically responsive and nonresponsive strains of mice, TCDD administration induced the four linked monooxygenase activities and the formation of cytochrome P_1-450 (10). Thus the so-called genetically nonresponsive mice do respond to the right stimulus and do possess the structural and regulatory genes necessary for the expression of aryl hydrocarbon hydroxylase. The defect in the nonresponsive mice appears to be a failure to recognize the usual, less potent aromatic hydrocarbons used as inducing drugs. We postulated that the site to which BNF and MC bind to initiate the induction of aryl hydrocarbon hydroxylase is defective in genetically nonresponsive mice, with a diminished affinity for these aromatic hydrocarbons.

There appear to be two possibilities to explain the induction of hydroxylase activity by TCDD in nonresponsive mice. Either TCDD interacts with a binding site different from that to which MC binds, a receptor[3] that is not defective and is similar in both responsive and nonresponsive mice, or TCDD acts at the same binding site as MC, but the greater potency of TCDD—and hence presumably its greater binding affinity—permits receptor saturation and full expression of enzyme activity in nonresponsive mice. One should be able to distinguish between these two possibili-

[1] *Induction* of aryl hydrocarbon hydroxylase activity is used to denote a relative increase in enzyme activity, measured *in vitro* by the increased rate of formation of 3-hydroxybenzo[*a*]pyrene. At present we cannot distinguish whether the increased hydroxylase activity that accumulates is the result of an increased rate of synthesis of the enzyme *de novo*, an increased activation of the enzyme from pre-existing moieties, or both these processes.

[2] The abbreviations used are: MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BNF, β-naphthoflavone.

[3] *Receptor* is used in the classical pharmacological sense, to denote the cellular element with which the drug must combine to initiate its response—in this case, induction of aryl hydrocarbon hydroxylase. The properties characterizing a drug-receptor interaction have all been demonstrated for TCDD (9): potency of the drug, the production of a graded biological response, which is dose-related, and the chemical and biological specificity of the drug-receptor interaction.

ties experimentally: the first predicts that genetically responsive and nonresponsive mice should have approximately the same sensitivity to TCDD; the second hypothesis predicts that all nonresponsive mice will be less sensitive to TCDD.

In this report we present evidence that genetically nonresponsive mice require a greater dose of TCDD than responsive mice for the induction of hepatic aryl hydrocarbon hydroxylase; that is, the dose-response curve is shifted to the right.

METHODS

NADPH, bovine serum albumin, and benzo[*a*]pyrene were purchased from Sigma Chemical Company. The benzo[*a*]pyrene was recrystallized from warm benzene by the addition of cold methanol. 3-Methylcholanthrene was purchased from Sigma Chemical Company and K & K Laboratories.

Dr. Harry Gelboin, National Cancer Institute, generously provided a sample of 3-hydroxybenzo[*a*]pyrene, and Dr. Albert Pohland, Food and Drug Administration, Washington, D. C., generously provided a sample of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (lot F-883) (11). *Special precautions were used in handling TCDD*, as previously outlined (12), *including the use of disposable gloves and benchtop coverings, and as much disposable plastic ware as possible. Glassware was routinely cleaned with potassium dichromate–sulfuric acid solution.*

Animals. All mice were obtained from the Jackson Laboratory, Bar Harbor, Me. The mice were housed in plastic cages with hardwood bedding, exposed to a day-night light cycle of 12 hr, and given food and water ad libitum.

In experiments examining the log-dose response curves, every attempt was made to minimize variability; homozygous responsive strains were tested simultaneously with homozygous nonresponsive strains or heterozygous hybrids which were matched for age and sex. Unless otherwise stated, the mice were immature females 4–9 weeks of age. The exceptions (see legends to Figs. 3 and 4 and Table 1) were inbred or hybrid mice that were difficult to obtain, and some variation in age and mixture of sexes were unavoidable.

The mice were injected intraperitoneally with *p*-dioxane (0.4 ml/kg) or TCDD in the same volume of solvent, using a Hamilton microsyringe. MC (80 mg/kg) was administered in corn oil (8 ml/kg). The mice were weighed and dosed on the basis of weight to the nearest 0.1 g. All animals were fasted for 10–16 hr before death.

Enzyme activity. Aryl hydrocarbon hydroxylase activity was assayed as described previously (9). The assay was performed on the 10,000 × *g* supernatant fraction of the liver rather than on the microsomal fraction, because of the large number of individual determinations. Each assay was performed in duplicate; duplicates usually varied less than 10%. Under the conditions employed, the assay was linear with enzyme concentration and time and at the pH optimum.

One unit of aryl hydrocarbon hydroxylase activity was defined as the amount of enzyme catalyzing the formation of hydroxylated product per minute at 37° equivalent to 1 pmole of 3-hydroxybenzo[*a*]pyrene. Activity was expressed as units per milligram of liver, wet weight.

RESULTS

The log dose-response curves for the induction of hepatic aryl hydrocarbon hydroxylase activity by TCDD in six strains of inbred mice are shown in Fig. 1A–C. TCDD induces enzyme activity in inbred strains of mice which are responsive to MC (C57BL/6J, BALB/cJ, A/J) as well as strains which are nonresponsive to MC (DBA/2J, AKR/J, SJL/J). However, nonresponsive strains were less sensitive to TCDD; that is, a larger dose of TCDD was required than in the sensitive strains to produce a comparable degree of induction.

In Fig. 2 the same data are presented as fractional responses to eliminate strain differences in the basal enzyme activity and the maximally induced activity. This was accomplished by equating the mean control activity to zero, and the mean maximally induced activity to 1.0, and calculating the fractional response of the

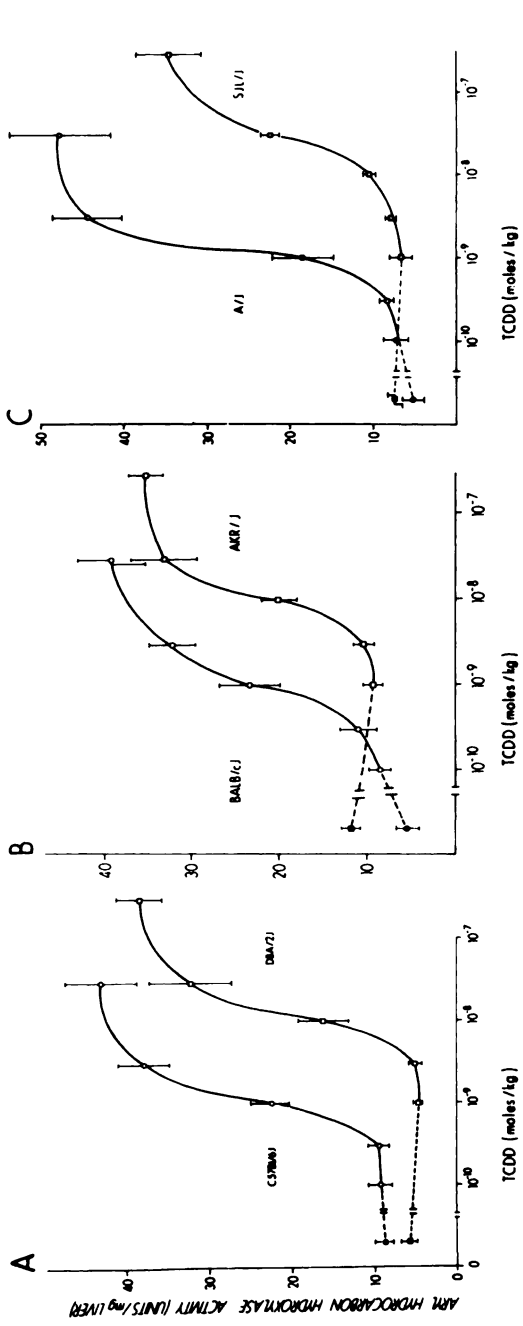

FIG. 1. *Log dose-response curves for induction of aryl hydrocarbon hydroxylase by TCDD in six inbred strains of mice*

The mice were injected intraperitoneally with *p*-dioxane or various doses of TCDD in this solvent; 24 hr later they were killed and hepatic anzyme activity was assayed. ○, genetically responsive mice; □, nonresponsive mice. The values of the control animals (solvent-injected), ●, and ■, are connected to the dose-response curve by dashed lines. A, C57BL/6J and DBA/2J; B, BALB/cJ and AKR/J; C, A/J and SJL/J. The points are the means ± standard errors of four or five animals.

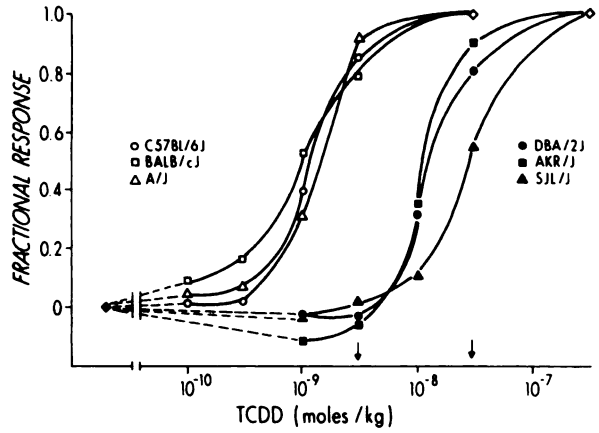

FIG. 2. *Log dose response curves for induction of aryl hydrocarbon hydroxylase transformed to fractional responses*

The data are those in Fig. 1, and the mean absolute enzyme activities have been converted to fractional responses, as explained in the text.

intermediate values for each strain.[4] The most useful parameter of sensitivity of the individual mouse strains is that dose of TCDD which elicits 50% of the maximal induction of hepatic aryl hydrocarbon hydroxylase, the ED_{50}. As seen in Fig. 2, the genetically responsive strains have ED_{50} values of about 1 nmole/kg and are about 10 times more sensitive to TCDD than the genetically nonresponsive strains ($ED_{50} \geqq$ 10 nmoles/kg).

The data in Figs. 1 and 2 were obtained 24 hr after the administration of TCDD, at which time the response observed was about half the maximum response (which is reached by 48 hr). Despite the submaximal enzyme induction at this earlier time, the ED_{50} values for different strains were very similar at 24 and 48 hr (Table 1).

In previous investigations of the genetic expression of aryl hydrocarbon hydroxylase the presence or absence of enzyme induction following a single large dose of BNF or MC was used to classify inbred strains as responsive or nonresponsive to aromatic hydrocarbons (3, 4). If the findings in Fig. 2 are generally applicable to other inbred strains of mice, it should also be possible to classify mice on the basis of their sensitivity to TCDD at a dose of 3 nmoles/kg (marked by an arrow in Fig. 2). Inbred strains induced by MC should also respond to this dose of TCDD. Mice that are nonresponsive to MC should not be induced by TCDD at a dose of 3 nmoles/kg but should respond to a 10-fold higher dose (marked by a second arrow in Fig. 2).

Fourteen strains of inbred mice were tested for their response to MC and TCDD administered 24 hr before death (Fig. 3). Each strain was divided into four treatment groups: control (*p*-dioxane); MC 0.3 mmole/kg; TCDD, 3 nmoles/kg; and TCDD, 30 nmoles/kg. The seven inbred strains on the left side of Fig. 3 were responsive; both MC and TCDD, at 3 nmoles/kg, induced hepatic aryl hydrocarbon hydroxylase activity. The other seven strains (on the right in Fig. 3) were nonre-

[4] Transformation of the absolute values of enzyme activity to fractional responses assumes that the maximal response observed is the true maximum. This assumption appears justified in C57BL/6J, BALB/cJ, A/J, DBA/2J, and AKR/J mice, because a 10-fold increase in the dose of TCDD from the next-to-highest to the highest dose produced only a small increase in enzyme activity. That is, the response formed a plateau. However, in the SJL/J animals, there was a substantial increase in enzyme activity when the dose of TCDD was increased from 30 to 300 nmoles/kg, suggesting that the true maximal response had not yet been reached. If this was the case, the actual log dose-response curves would be to the right of the observed curve and the true ED_{50} would be greater.

TABLE 1

Sensitivity of various inbred and hybrid strains of mice to TCDD

Inbred and hybrid mice were injected with *p*-dioxane or TCDD, and hepatic aryl hydrocarbon hydroxylase activity was assayed 24 or 48 hr later. Details on most of the inbred and hybrid strains are found in the legends to figures. The C3H/HeJ and C3D2F$_1$/J mice were 7-week-old females, with four to six animals per dose level and six or seven dose levels, respectively. The AKR/J B6AKF$_1$, and C57BL/6J (in one experiment) were 6-week-old mice, both male and female, with five or six animals per dose level and seven, seven, and six dose levels of TCDD, respectively. Each value is the mean ± standard error of four to six mice.

Mouse strain	ED_{50}	Aryl hydrocarbon hydroxylase activity	
		Control	Maximally induced
	nmoles/kg	*units/mg liver*	
24-hr induction			
C57BL/6J	1.2	8.9 ± 1.2	42.7 ± 4.3
BALB/cJ	1.0	5.6 ± 1.1	39.2 ± 3.9
A/J	1.2	5.1 ± 1.4	47.9 ± 6.2
DBA/2J	13.8	5.9 ± 1.0	38.1 ± 2.7
AKR/J	11.0	11.9 ± 1.1	35.2 ± 1.9
SJL/J	25.0	7.4 ± 0.9	34.8 ± 4.0
48-hr induction			
C57BL/6J	1.7	11.3 ± 0.8	87.9 ± 4.6
	1.7	11.1 ± 0.5	103.6 ± 4.8
C3H/HeJ	2.7	4.6 ± 0.9	64.7 ± 5.8
DBA/2J	16.0	7.9 ± 1.6	69.9 ± 12.1
AKR/J	9.2	13.0 ± 1.6	67.7 ± 3.5
B6D2F$_1$/J[a]	4.2	9.1 ± 0.9	123.8 ± 12.9
C3D2F$_1$/J[b]	5.1	7.8 ± 1.2	90.7 ± 7.6
B6AKF$_1$/J[c]	1.9	10.5 ± 1.2	88.8 ± 9.7

[a] (C57BL/6J female × DBA/2J male)F$_1$.
[b] (C3H/HeJ female × DBA/2J male)F$_1$.
[c] (C57BL/6J female × AKR/J male)F$_1$.

sponsive; neither MC nor the low dose of TCDD significantly increased the enzyme activity. However, a 10-fold higher dose of TCDD produced substantial induction of hydroxylase activity.

Several studies have shown that aromatic hydrocarbon responsiveness is inherited as a simple autosomal dominant trait in the crosses and back-crosses between the prototype strains, C57BL/6 and DBA/2 (2, 4). Thus the heterozygous offspring of these two strains, B6D2F$_1$, are fully inducible by a single large dose of MC and are indistinguishable from the induced responsive parental strain, C57BL/6. If the genetic defect in nonresponsive mice is in the structure of a receptor protein, it should be possible to distinguish the heterozygous offspring from the C57BL/6 parents by their sensitivity to induction by TCDD, because altered binding affinity to the putative receptor should be apparent even if the resultant induction were maximal (i.e., phenotypically "dominant"). The log dose-response curves for the induction of aryl hydrocarbon hydroxylase by TCDD in C57BL/6J, B6D2F$_1$/J, and DBA/2J mice are shown in Fig. 4. The enzyme activities were measured 48 hr after TCDD administration, and it will be noted that the enzyme activities in the two parent strains are higher in Fig. 4A than those shown in Fig. 1A. However, the ED_{50} values for C57BL/6 and DBA/2 mice were very similar at 24 and 48 hr (Table 1). The F$_1$ heterozygous mice were intermediate in their sensitivity. This is more evident when the data are plotted as the fractional response (Fig. 4B). The maximum enzyme activity induced in the hybrid B6D2F$_1$/J was higher than that observed in either of the inbred parent strains. This heterosis was also observed in the C3D2F$_1$/J hybrid. In the C3H/HeJ vs. DBA/2J cross, the heterozygote C3D2F$_1$/J showed an intermediate or additive expression of aryl hydrocarbon hydroxylase when tested with MC at any dose (13, 14). However, in response to TCDD, the C3D2F$_1$/J was fully expressed and the absolute activity was higher than in either of the parent strains.

In Fig. 4 the aryl hydrocarbon hydroxylase activity in C57BL/6J mice falls as the dose of TCDD is increased from 6 to 60 nmoles/kg. While this decrease in the response is substantial, it is not statistically significant, nor have we observed any significant decrease of enzyme activity with high doses of TCDD administered to other strains. We are unaware of any toxic effects of TCDD *in vivo* in the first 48 hr after administration.

The ED_{50} (to TCDD) and the maximal inducibility of each of the inbred and hybrid mice tested are presented in Table 1. Several points are of note. The estimates

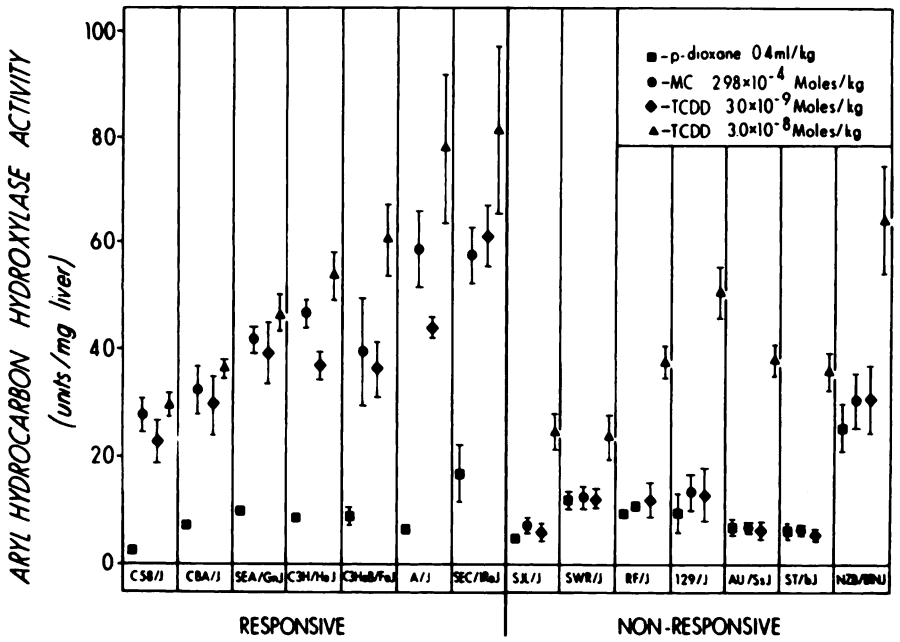

FIG. 3. *Screening of 14 inbred strains of mice for responsiveness to aromatic hydrocarbons*

Twelve mice from each strain were divided into four treatment groups: ■, *p*-dioxane-injected controls; ●, MC, 298 μmoles/kg in corn oil, ◆, TCDD, 3 nmoles/kg in *p*-dioxane; ▲, TCDD, 30 nmoles/kg in *p*-dioxane. The mice in each inbred strain were females of identical age (varying from 4 to 8 weeks) with the following exceptions: SEA/GnJ consisted of 7- and 9-week-old females; Au/SsJ, 9–13-week-old males and females; and ST/bJ, 6-week-old males.

of the ED_{50} at 24 and 48 hr are in good agreement in every strain examined despite the greater induction at the later time interval. The three hybrids studied, $B6D2F_1/J$, $C3D2F_1/J$, and $B6AKF_1/J$, showed a sensitivity to TCDD that was intermediate between those of their homozygous responsive and homozygous nonresponsive parents. However, the difference in sensitivity of the $B6AKF_1/J$ and C57BL/6J parent strains is not significant. All responsive strains had ED_{50} values of about 1 nmole/kg, which is similar to those previously reported for Sprague-Dawley rats (ED_{50}, approximately 0.85 nmole/kg) and for chick embryos [ED_{50}, approximately 0.4 nmole/kg (12)], and in sharp contrast to the diminished sensitivity of nonresponsive inbred mouse strains.

DISCUSSION

We have previously shown that the administration of TCDD induces aryl hydrocarbon hydroxylase activity in inbred strains of mice which fail to respond to the usual aromatic hydrocarbons (10). In the present communication we report that the dose of TCDD required to induce hepatic enzyme activity to half the maximal response (ED_{50}) was approximately 1 nmole/kg in responsive mice, at least 10 nmoles/kg in nonresponsive mice, and intermediate in the heterozygous offspring of responsive and nonresponsive parents. Thus the phenotypic characteristics of aromatic hydrocarbon responsiveness, when tested in the usual manner with MC, were either present or absent; however, when this trait was examined with the more potent inducer, TCDD, responsiveness appeared to be only a greater degree of sensitivity.

In the prototype cross C57BL/6J and DBA/2J, aromatic hydrocarbon responsiveness is inherited in a simple autosomal mode and the locus controlling this expression is termed the Ah locus (4, 5). It is important to ask the nature of the Ah

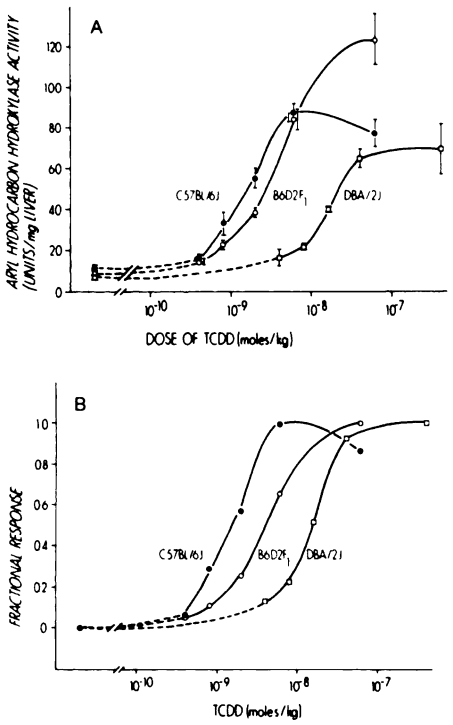

FIG. 4. *Log dose-response curves for induction of aryl hydrocarbon hydroxylase by TCDD in C57BL/6J, DBA/2J, and the heterozygous offspring B6D2F$_1$/J*

Mice were injected with *p*-dioxane or TCDD and killed 48 hr later, and hepatic aryl hydrocarbon hydrolase activities were assayed. All mice were 6-week-old females. A. Absolute enzyme activities; each point is the mean ± standard error of four to six mice. B. The mean enzyme activities were converted to fractional responses.

locus, and the nature of the mutation in nonresponsive mice. Often it is difficult to distinguish a mutation in a structural gene from one in a regulatory gene, because both mutations may give a similar phenotypic expression.

The Ah locus might represent the structural gene(s) which codes for aryl hydrocarbon hydroxylase and cytochrome P_1-450, and the phenotype observed in nonresponsive mice would result from the expression of a faulty gene product with little or no enzymatic activity and none of the spectral properties that characterize cytochrome P_1-450. Nebert and Gielen (5) summarized the evidence against a structural gene mutation as the explanation for the nonresponsive phenotype. The most incontrovertible evidence is that the administration of TCDD to nonresponsive mice induces aryl hydrocarbon hydroxylase activity and cytochrome P_1-450 indistinguishable from those expressed in responsive mice (10).

Thus nonresponsive mice have the structural and regulatory genes necessary for the expression of aryl hydrocarbon hydroxylase activity and, given the appropriate stimulus, they will respond. The defect in these mice is such that the usual aromatic hydrocarbons, such as MC or BNF, fail to evoke a response. These findings suggest that the mutation in nonresponsive mice results in an induction receptor[5] with diminished affinity for aromatic hydrocarbons. There appear to be two possible explanations for the enzyme induction produced by TCDD in the nonresponsive mice: either TCDD acts on a site different from the receptor for MC, a site which is unaffected by the mutation in the Ah locus of nonresponsive mice, or TCDD acts at the same receptor as MC. Despite the diminished affinity of the receptor for both drugs, the greater potency of TCDD, and hence its presumably greater binding affinity, permits sufficient receptor occupation to initiate enzyme induction. The first hypothesis predicts that responsive and nonresponsive mice will be equally sensitive to TCDD; the second predicts that nonresponsive mice will be less sensitive to TCDD than responsive strains. The data (Figs. 2 and 3) clearly support the second hypothesis.

Further evidence that TCDD and other aromatic hydrocarbons act on the same receptor comes from studies of heterozygous F_1 mice. The hybrid mice B6D2F$_1$/J

[5] The term *induction receptor* denotes the product of a regulatory gene that combines with a small molecule to initiate gene expression. One might also refer to the receptor as an apoinducer or apoderepressor. We prefer induction receptor because it is not known whether the expression of aryl hydrocarbon hydroxylase is under positive or negative control, nor how many steps intervene between the drug-receptor interaction and the expression of the structural gene for enzyme activity.

and C3D2F$_1$/J are less sensitive to induction than their responsive homozygous parents, C57BL/6J and C3H/HeJ, respectively. The log dose-response curves for these heterozygous mice are shifted to the right of those of the parent strains when TCDD is the inducing drug (Fig. 4 and Table 1) and also when BNF is the inducing drug (14).

The evidence available is consistent with the hypothesis that the Ah locus is a regulatory gene whose product is the induction receptor for the expression of the induced form of aryl hydrocarbon hydroxylase and cytochrome P$_1$-450. This receptor is the common binding site for MC, BNF, and TCDD. The mutation in the nonresponsive mice results in a receptor with a diminished affinity for these inducing drugs. Conceivably, nonresponsive mice could metabolize aromatic hydrocarbons or TCDD more rapidly or have a diminished cellular uptake of these drugs; either process would reduce the effective intracellular concentration of these compounds. Nebert and Bausserman (15) have found that the uptake and metabolism of the polycyclic hydrocarbon benz[*a*]anthracene were similar in cell cultures derived from responsive and nonresponsive fetal mice, and that cellular uptake occurred by passive diffusion. Vinopal and Casida (16) were unable to detect any metabolism of tritiated TCDD *in vivo* over a 20-day period in mice, or *in vitro* by mouse liver microsomes. TCDD, like benz[*a*]anthracene, is a nonpolar xenobiotic, and we are unaware of any report of facilitated or active transport for nonpolar compounds. Thus the data available do not support any significant differences in the fate of the inducing drugs in responsive and nonresponsive mice.

In the original investigations of aromatic hydrocarbon responsiveness on crosses of C57BL/6 and DBA/2 mice, the trait was shown to segregate in a simple autosomal dominant mode (2-4). Robinson *et al.* (8), in an extensive examination of crosses and back-crosses among 12 inbred strains of mice, reported that the inheritance pattern of aromatic hydrocarbon responsiveness (tested with BNF) is controlled by at least two nonlinked loci. We have had limited experience with crosses that behave other than in the dominant mode, but we have found no inbred or hybrid mice in which aryl hydrocarbon hydroxylase is not induced by TCDD; the mice differ only in their sensitivity to the drug.

The induction of aryl hydrocarbon hydroxylase activity has been studied with increasing interest as a mode of gene expression. The Ah locus appears to be a regulatory locus for hydroxylase activity (5), and the data presented in this communication suggest that the mutation in the Ah locus in nonresponsive mice results in the formation of a defective induction receptor with diminished affinity for inducing drugs. A similar receptor mutation resulting in a regulatory failure has been reported in familial hypercholesterolemia (17).

In contrast to other aromatic hydrocarbon inducers, TCDD has several distinct properties which will make it a valuable tool in future studies on the induction of aryl hydrocarbon hydroxylase activity: resistance to metabolic degradation, prolonged duration of action, and extraordinary potency (which implies a high affinity for the induction receptor).

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