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Influence of the *Ah* Locus on the Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on the Hepatic Epidermal Growth Factor Receptor

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SUMMARY

The present studies examine whether the Ah receptor mediates the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the binding capacity of the hepatic epidermal growth factor (EGF) receptor in congenic strains of C57BL/6J mice that differ only at the *Ah* locus. The *Ah* locus is believed to encode the Ah receptor, which mediates the induction of cytochrome P4501A1 by TCDD and appears to mediate many of the toxic effects of TCDD. TCDD produced an 80–90% decrease in the maximum binding capacity (both high and low affinity sites) of the hepatic EGF receptor in female Ah-responsive ($Ah^{b/b}$) and *Ah*-nonresponsive ($Ah^{d/d}$) C57BL/6 mice. However, the ED_{50} for the effects of TCDD on the binding capacity of the EGF receptor was 10-fold higher in the Ah-nonresponsive mice, compared with the Ah-responsive mice (7 versus 0.7 μg/kg). TCDD did not affect the hepatic content of two EGF receptor mRNA transcripts (10 and 6 kb), indicating that the effects on the EGF receptor are not pretranslational. Similarly, TCDD did not affect the hepatic content of mRNA for transforming growth factor-α, an alternate ligand for the EGF receptor that is synthesized in the liver. In contrast, TCDD markedly increased the hepatic content of the mRNA for cytochrome P4501A1, which is known to be regulated transcriptionally by TCDD. The ED_{50} for this effect was 10-fold higher in Ah-nonresponsive mice than in Ah-responsive mice (13 versus 1.3 μg/kg). This study indicates that the effects of TCDD on EGF receptor ligand binding are mediated by the Ah receptor. However, unlike the effect of TCDD on cytochrome P4501A1, the effects of TCDD on the EGF receptor do not involve changes in the levels of the mRNA for this protein or changes in the mRNA for transforming growth factor-α, an alternate ligand for the EGF receptor.

TCDD is one of the most toxic environmental chemicals known. It produces a variety of biochemical and toxic responses, including induction of P4501A1, chloracne, immunotoxicity, teratogenicity, liver lesions, hepatic porphyria, carcinogenicity, and a slow wasting syndrome that is followed by the death of the animal (1). Although the mechanism of toxicity is not completely understood, it is believed that most of the biological responses to TCDD are mediated through an intracellular protein, the Ah receptor. The affinities of various halogenated dibenzodioxin and dibenzofuran congeners for this receptor correlate well with their toxicities and their abilities to induce P4501A1. The Ah receptor-TCDD complex has been shown to bind with high affinity to specific enhancer regions in the upstream region of the structural gene for P4501A1 and to increase transcription of this target gene (2, 3). It has been suggested that the majority of the other biological effects of TCDD may be mediated through changes in transcription of other target genes (1).

TCDD has been shown to decrease the binding affinity of a number of other endogenous receptors, including the EGF receptor, for their ligands the glucocorticoid receptor, and the estrogen receptor (4–6). Many of these growth factors, including EGF and TGF-α, are important in cell differentiation (7–9). TCDD is known to affect proliferation and differentiation in a number of target tissues, including skin, liver, and the palate of the mouse embryo (10–12). Therefore, the effects of TCDD on the EGF receptor might be involved in some of the biological effects of TCDD. The mechanism by which TCDD decreases the binding capacity of the EGF receptor and that of receptors for other endogenous ligands is not known.

Certain strains of mice have been shown to be 10-fold more responsive (Ah-responsive, prototype C57BL/6J) to the inductive effects of TCDD on P4501A1 than other strains of mice (Ah-nonresponsive, prototype DBA/2) (1, 13). Responsiveness

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ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; EGF, epidermal growth factor; SSC, standard sodium citrate; SDS, sodium dodecyl sulfate; TGF-α, transforming growth factor-α; EROD, ethoxyresorufin *O*-deethylase; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; P4501A1, cytochrome P4501A1; kb, kilobases; ED_{50}, 50% effective dose; PIPES, 1,4-piperazinediethanesulfonic acid.

appears to be mediated by a single gene locus, known as the *Ah* locus. The *Ah* locus is thought to encode the structural gene for the Ah receptor, and the defect in nonresponsive mice appears to be a defective Ah receptor with a lower affinity for TCDD (14). Congenic strains of C57BL/6J mice have become available that differ only at the *Ah* locus (and possibly a limited number of closely linked genes). The present studies examined whether the Ah receptor mediates the effects of TCDD on the hepatic EGF receptor, using these congenic strains of mice. We also examined whether the decrease in EGF receptor binding involves a pretranslational effect on the mRNA for the EGF receptor or whether TCDD affects the mRNA for TGF-α, a ligand for the EGF receptor that is synthesized in the liver (9).

Materials and Methods

Chemicals. Phenylmethylsulfonyl fluoride, PIPES, and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, MO), leupeptin from Boehringer Mannheim Biochemicals (Indianapolis, IN), and EGF (culture grade) from Collaborative Research (Bedford, MA). Denhardt's solution, salmon sperm DNA, yeast tRNA, and dextran sulfate were obtained from 5 Prime-3 Prime (West Chester, PA). The genomic clone containing a fragment of the mouse EGF receptor (pME 2.0) was a generous gift from Dr. Mien-chie Hung (The University of Texas System Cancer Center, M. D. Anderson Hospital, Houston, TX). The cDNA probe for TGF-α was generously provided by Dr. David Lee (University of North Carolina, Chapel Hill, NC). The ^{32}P-labeled actin probe was purchased from Oncor (Gaithersburg, MD) and the mouse P4501A1 cDNA probe from the American Type Culture Collection (Rockville, MD). ^{125}I-EGF (>95% pure) was from Diagnostic System Laboratories (Webster, TX). All other chemicals were reagent grade.

Animals. Congenic female wild-type Ah-responsive C57BL/6J (Ah$^{b/b}$) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Congenic female Ah-nonresponsive (Ah$^{d/d}$) mice were supplied by Dr. Poland (University of Wisconsin, Madison, WI) and do not differ from normal C57BL/6J (Ah$^{b/b}$) mice in any of 32 biochemical markers examined (15). The congenic C57BL/6N Ah$^{d/d}$ mice were originally derived by Dr. Nebert (National Institutes of Health), who bred the Ah-nonresponsive phenotype (*Ah^{d}* allele) of DBA/2N mice into a C57BL/6N background by back-cross/inter-cross. They were B6N.D2N-Ahd (NE13) when received from Dr. Nebert and were then bred into The Jackson Laboratory (C57BL/6J) background for seven back-cross/inter-cross cycles (B6J.D2-Ah$^{d/d}$ (NE7) by Dr. Poland, as previously described (16). These congenic mice were then maintained at Research Triangle Institute. Ah$^{b/b}$ mice were received 4–5 weeks before the start of the experiment and Ah$^{d/d}$ mice at least 1 week before the start of the experiment (10–11 weeks of age). Ah$^{b/b}$ and Ah$^{d/d}$ mice were randomized using a table of random numbers and housed, four or five/cage, in separate rooms under identical conditions, at constant temperature (70 ± 5°F), humidity (50 ± 5%), and lighting (12/12-hr), and received food and water *ad libitum*.

Membrane preparations. Hepatic plasma membranes were isolated by a Percoll gradient technique described by Inui *et al.* (17). Mice were anesthetized with CO_2 and sacrificed between 9:00 a.m. and 12 noon. Each liver was finely minced and homogenized in SET buffer (10 mM Tris, pH 7.5, 1 mM EDTA, 0.25 M sucrose) containing protease inhibitors (20 μg/ml leupeptin and 170 μg/ml phenylmethylsulfonyl fluoride), using 20 passes of a Dounce homogenizer. The homogenate was then centrifuged at 2,600 × *g* for 15 min at 4°, and the resultant supernatant was centrifuged at 20,000 × *g* for 20 min at 4°. The pellet was resuspended in 20% Percoll in SET buffer with protease inhibitors and then fractionated by centrifugation at 63,000 × *g* for 30 min. The band of plasma membranes located one third of the distance from the top of gradient was collected and washed twice with 0.1 M phosphate buffer (pH 7.3), by centrifugation at 160,000 × *g* for 30 min, to remove residual Percoll. The plasma membranes were then resuspended in the same buffer and stored at −70° until assayed for EGF receptor binding. Protein concentrations were assayed using the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL), with bovine serum albumin as standard.

Binding assay. The ^{125}I-EGF receptor binding assay was performed under equilibrium conditions (18). The hepatic membranes (20 μg) were incubated for 1 hr at 22° with 50 mM phosphate buffer (pH 7.3) containing 0.1% bovine serum albumin and ^{125}I-EGF (0.02 to 1 nmol), in a final volume of 200 μl. Replicate tubes containing 200 nmol of unlabeled EGF were used to estimate nonspecific binding. After incubation, 3 ml of ice-cold 50 mM phosphate buffer containing 0.1% bovine serum albumin were added, to terminate ligand binding, and the plasma membrane-bound ^{125}I-EGF was then separated from the unbound ligand by passage of the sample through a glass fiber filter, using a Millipore apparatus (Whatman, England). Specific binding (SB) was calculated as the difference between total and nonspecific binding (fmol of ^{125}I-EGF/mg of protein). Scatchard plots were fitted using the method of least squares (19). The dissociation constant (K_d) and maximum ligand binding capacity (B_{max}) of the high affinity binding site were derived directly from the inverse of the slope and the *x*-intercept, respectively, of the first linear portion of the curve, using linear regression analysis. Using the same method, the K_d and the B_{max} for the low affinity binding site were derived from the inverse of the slope and the *x*-intercept, respectively, of the second linear portion of the curve.

Isolation of mRNA and Northern blot hybridization. Total RNA was isolated from 1 g of liver in 4 M guanidium thiocyanate, by the procedure described by Chomczynski and Sacchi (20). Poly(A)$^+$ RNA was isolated by oligo(dT) chromatography (21). Aliquots (both 2 and 6 μg) of poly(A)$^+$ RNA were electrophoresed on 1% agarose-2.2 M formaldehyde gels and transferred overnight to Nytran membranes (Schleicher & Schuell, Keene, NH). The filters were baked at 85° for 2 hr. The filters were hybridized overnight with a nick-translated ^{32}P-labeled actin probe, at 42°, in a 50% formamide, 5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 2.5 × Denhardt's (1 × Denhardt's is 0.1 g/ml Ficoll, 0.1 g/ml polyvinylpyrrolidone, and 0.1 g/ml bovine serum albumin), 20 mM sodium phosphate buffer, pH 6.5, containing 8% dextran sulfate, 0.5% SDS, and 150 μg/ml denatured salmon sperm DNA. Filters were washed three times, for 30 min each time, at 65° in 1 × SSC, 0.5% SDS. Filters were then autoradiographed, and the mRNAs were quantitated by scanning with an LKB Ultroscan laser densitometer (LKB Instruments). The integrated values of the peaks (or in some cases peak heights) were plotted as mean absorbance/mg of poly(A)$^+$ RNA.

The filters were then stripped by heating 0.1 × SSC, 0.1% SDS, to 95°, pouring it over the blot, and shaking until the solution reached room temperature. A ^{32}P-labeled antisense EGF receptor RNA was generated from pME 2.0, using a Riboprobe system (Promega, Madison, WI). Filters were prehybridized for 2 hr at 55° in 50% formamide, 0.8 M NaCl, 2.5 × Denhardt's solution, 50 mM phosphate, pH 7.0, 1 mM EDTA, 250 μg/ml salmon sperm DNA, 500 μg/ml yeast tRNA, as previously described (22). They were then hybridized with the ^{32}P-EGF receptor riboprobe overnight, washed four times, for 30 min each time, at 65° in 0.1 × SSC, 1 mM EDTA, 0.5% SDS, autoradiographed, and scanned as described above. Filters were then stripped, rehybridized with a ^{32}P-labeled TGF-α cDNA as described for actin, and autoradiographed. Finally, filters were stripped and rehybridized overnight with a ^{32}P-labeled cDNA probe for mouse P4501A1, in 6 × SSC, 4 × Denhardt's, 0.5% SDS, 100 μg/ml DNA, at 60°. Filters were washed two times, for 30 min each time, at 60° in 0.2 × SSC, 0.1% SDS, autoradiographed, and scanned as described above. A standard curve for each RNA was determined using different amounts of poly(A)$^+$ RNA, and all samples were quantitated by laser densitometry within the linear portion of the curve. All values were normalized by hybridization with the actin probe.

Statistics. The method of least squares was used to fit the EGF binding data in Scatchard plots. Data were expressed as means ±

standard errors. Values were assessed as both actual values and as percentages of control. Analysis of variance procedures and linear regression trend tests were employed to assess the differences between congenic strains and the dose effect (23). Pairwise comparisons were made by Dunnett's test (versus controls) or Fischer's least significant difference test (24). To fit P4501A1 mRNA levels to the dose-response curves, the nonlinear least squares method was used and significance was assessed with F tests.

Results

Effects of TCDD on hepatic EGF receptor binding. Scatchard analysis of EGF binding to hepatic plasma membranes of female Ah-responsive ($Ah^{b/b}$) C57BL/6J mice (Fig. 1) revealed two classes of displacable ^{125}I-EGF binding sites. High affinity binding sites exhibited a dissociation constant (K_d) of approximately 0.2 nM and an apparent maximum binding capacity (B_{max}) of approximately 500 fmol/mg of protein. The low affinity binding sites had a K_d of approximately 1 nM and an apparent B_{max} of ~2000 fmol/mg of protein. TCDD (30 μg/kg) decreased the B_{max} for both the high and the low affinity binding sites to ~100 and 500 fmol/mg of protein, respectively, with no change in the K_d values. Time course studies indicated that the maximum binding capacity of the hepatic EGF receptor (both the high and low affinity binding sites) was decreased by 80–90%, 7 days after treatment of Ah-responsive mice with TCDD (30 μg/kg) (Fig. 2). Earlier time course studies had shown that hepatic EROD activity was also maximally induced between days 1 and 7 (24a). Therefore, dose-response studies were performed 7 days after TCDD treatment.

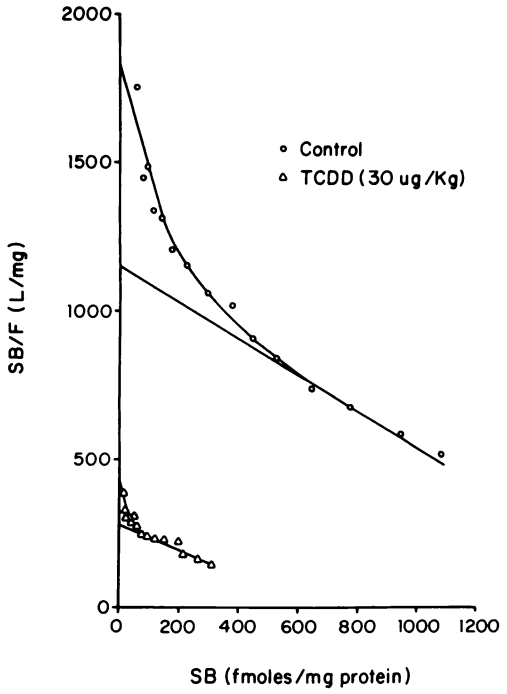

Fig. 1. Representative Scatchard analyses of ^{125}I-EGF binding to hepatic plasma membranes from female Ah-responsive ($Ah^{b/b}$) C57BL/6J mice, 7 days after treatment with a single dose of TCDD (30 μg/kg) (△) or the corn oil vehicle (○). Each *line* represents analysis of ^{125}I-EGF binding to plasma membranes from the liver of an individual animal. The apparent maximum binding capacity (B_{max}) and dissociation constant (K_d) for the high affinity binding site were derived directly from the x-intercept and the inverse of the slope, respectively, of the first linear portion of the curve, by linear regression analysis. The apparent B_{max} and K_d for the low affinity binding site were similarly derived from the second linear portion of the curve. For control liver membranes, the B_{max} values for the high and low affinity binding sites were 520 and 1920 fmol/mg of protein respectively, whereas the K_d values were ~0.2 and 2 nM, respectively. TCDD (30 μg/kg) decreased the B_{max} for the high and low affinity binding sites to 90 and 580 fmol/mg of protein but did not change either K_d value. SB, specific binding; F, concentration of (unbound) EGF in the binding assay.

There was a significant strain difference in the effects of TCDD on the binding capacity of both the high and low affinity sites of the EGF receptor in Ah-nonresponsive ($Ah^{d/d}$) mice, compared with Ah-responsive ($Ah^{b/b}$) mice ($p < 0.0001$) (Fig. 3). The ED_{50} values were approximately 10-fold higher in $Ah^{d/d}$ mice, compared with $Ah^{b/b}$ mice (~7 versus 0.7 μg/kg). This is similar to the 10-fold increase in the ED_{50} for induction of EROD activity (a P4501A1-mediated response) in $Ah^{d/d}$ mice in our laboratory (ED_{50} of 16 versus 1.6 μg/kg). The maximum binding capacity of the EGF receptor (both high and low affinity binding sites) appeared slightly higher in untreated $Ah^{d/d}$ mice than in untreated $Ah^{b/b}$ mice in the present experiment, but this difference was not statistically significant and did not occur in a second experiment with larger numbers of control animals ($n = 8$) [high affinity, 518 ± 72 ($Ah^{b/b}$) and 432 ± 39 ($Ah^{d/d}$); low affinity, 1864 ± 134 ($Ah^{b/b}$) and 1797 ± 145 fmol/mg of protein ($Ah^{d/d}$)]. The effects of TCDD on the EGF receptor were seen at doses that cause minimal systemic toxicity. Birnbaum *et al.* (25) reported that the 50% lethal dose for TCDD is ~159 μg/kg (no deaths at 100 μg/kg) in C57BL/6J $Ah^{b/b}$ mice and 3350 μg/kg in the congenic C57BL/6J $Ah^{d/d}$ mice. Approximately 5% depression of weight gain was seen at a dose of 100 μg/kg in $Ah^{b/b}$ mice in their study, indicating some toxicity at that dose, but there was no depression of weight gain at a dose of 50 μg/kg. Depression of body weight gain occurred in the congenic $Ah^{d/d}$ mice only at doses of ≥1600 μg/kg.

Effects of TCDD on hepatic EGF mRNA contents. Because TCDD is thought to act by affecting transcription of a battery of genes, we examined whether TCDD affected the hepatic content of the mRNA for the EGF receptor. Northern blot analysis demonstrated a major 6-kb mRNA for the EGF receptor and a minor 10-kb mRNA. A representative autoradiograph shows that TCDD had no effect on the 6- and 10-kb

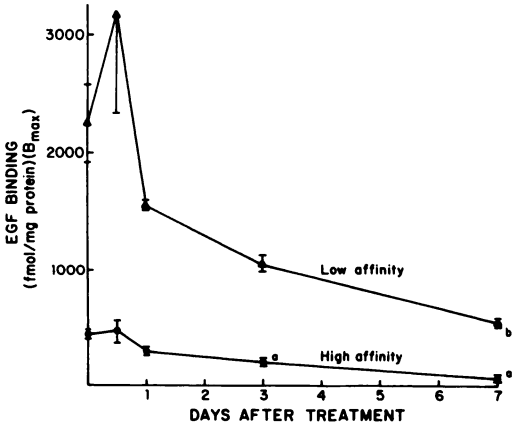

Fig. 2. Time course for the effect of TCDD on the B_{max} of both high (●) and low (▲) affinity binding sites of the EGF receptor in hepatic plasma membranes, following a single dose of TCDD (30 μg/kg) to Ah-responsive mice. Each *point* and *vertical bar*, mean ± standard error ($n = 4$). *a*, Significantly different ($p < 0.05$) from controls, using analysis of variance and Dunnett's test.

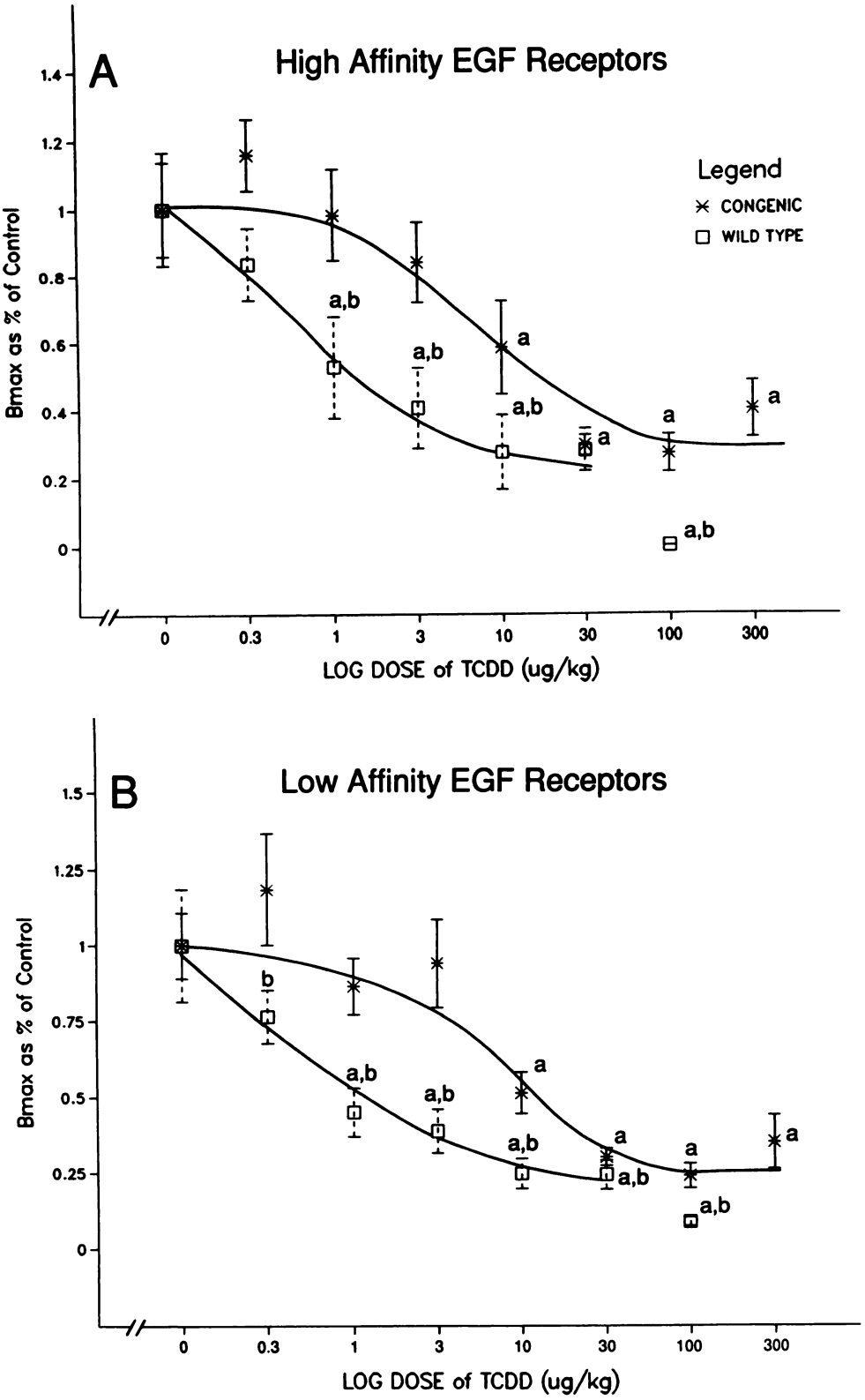

Fig. 3. Dose-response curves for the effects of TCDD on the maximum binding capacity (B_{max}) of the high affinity (A) and low affinity (B) binding sites of the EGF receptor in hepatic plasma membranes of wild-type Ah-responsive ($Ah^{b/b}$) and congenic Ah-nonresponsive ($Ah^{d/d}$) C57BL/6J mice, 7 days after treatment with varying doses of TCDD (0.3 to 300 μg/kg). The B_{max} values are expressed as a percentage of their respective control values. For high affinity sites, control values were 519 ± 72 ($Ah^{b/b}$ mice) and 789 ± 132 fmol/mg of protein ($Ah^{d/d}$ mice); for low affinity sites, 2083 ± 866 ($Ah^{b/b}$) and 2940 ± 322 fmol/mg of protein ($Ah^{d/d}$). Each *point* and *vertical bar*, mean ± standard error (n = 5). There was a statistically significant difference between strains for both the high (p = 0.0001) and low (p = 0.0001) affinity binding sites. The ED_{50} values (doses that gave half-maximal suppression of the B_{max}) were ~0.7 μg/kg in $Ah^{b/b}$ mice and 7 μg/kg in $Ah^{d/d}$ mice. *a*, Treatment group significantly different from controls ($p < 0.05$), by Dunnett's test. *b*, Significant difference between strains at this dose ($p < 0.05$), by Fisher's least significant difference test.

EGF receptor mRNAs in the two congenic strains of mice at any dose (Fig. 4). Laser densitometry scanning of the two bands confirmed that TCDD produced no significant changes in either of the two EGF receptor mRNA transcripts in either $Ah^{b/b}$ or $Ah^{d/d}$ mice (results of laser scans of the 6-kb band are shown in Fig. 6A). A time course for the effects of TCDD (30 μg/kg) on the EGF receptor mRNA also indicated that TCDD had no effect on the EGF receptor mRNA at 12 hr, 1 day, 3 days, or 7 days (data not shown).

EGF is not synthesized in the liver (26). However, TGF-α is an alternate ligand for the EGF receptor that is synthesized in the liver (8), and hepatic changes in this ligand could alter EGF

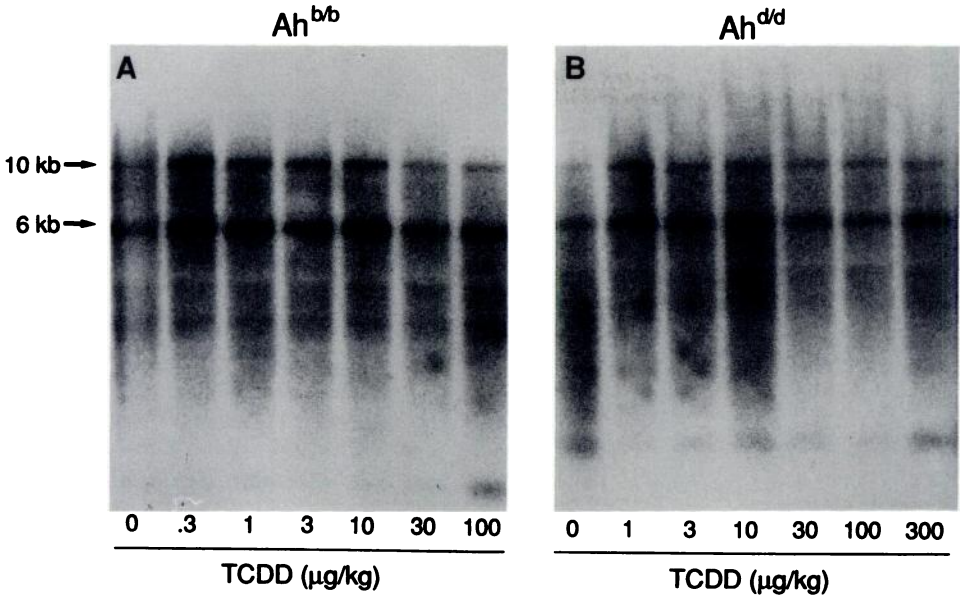

Fig. 4. Autoradiographs of Northern blots of the mRNA for the EGF receptor in Ah$^{b/b}$ (A) and Ah$^{d/d}$ (B) mice after treatment with various doses of TCDD. Animals were treated as described for Fig. 3. Poly(A)$^+$ RNA was prepared from liver, electrophoresed in 1% agarose-2.2 M formaldehyde gels, and transferred to a Nytran membrane. The filters were hybridized with an EGF receptor antisense RNA riboprobe, as described in Materials and Methods, and autoradiographed. Each blot contained 2 and 6 μg mRNA from two complete sets of wild-type and congenic mice. Each lane shown represents mRNA (2 μg) from a single animal. *Arrows*, the two EGF receptor mRNA transcripts (6 and 10 kb).

receptor levels by producing internalization and degradation (27). Therefore, we also examined the effects of TCDD on the hepatic content of TGF-α mRNA. Northern blot analysis showed the presence of a single hepatic TGF-α mRNA transcript of 4.8 kb, which was not affected by TCDD treatment (Fig. 5). The small variations noted here were consistent with small variations in actin mRNA. Laser densitometry scanning of the 4.8-kb band in autoradiographs of TGF-α mRNA from each of the animals indicated that TCDD did not significantly affect the level of this mRNA at any dose, when corrected for hybridization with actin (Fig. 6A). Moreover, the time course indicated that TCDD did not affect TGF-α mRNA at any time between 12 hr and 7 days after treatment (not shown).

In contrast to the absence of any effects of TCDD on EGF receptor mRNA or TGF-α mRNA, TCDD increased the 3.0-kb P4501A1 mRNA dramatically (Fig. 6B). The increase was nearly maximum 12 hr after TCDD treatment, and the mRNA remained maximally elevated for at least 7 days (data not shown). The dose-response curves in the two strains of congenic mice indicated that the ED_{50} for induction of P4501A1 mRNA was 10-fold lower in Ah-responsive mice (ED_{50} of 1.3 versus 13 μg/kg TCDD), as would be expected for a response known to be mediated by the Ah receptor. This increase is also consistent with the difference in the ED_{50} values for EROD induction in the congenic strains of Ah-responsive and Ah-nonresponsive mice (1.6 versus 15 μg/kg) (24).

Discussion

Several studies have shown that TCDD decreases the binding capacity of the EGF receptor for its ligand in both liver and isolated keratinocytes (6–11). EGF is a potent mitogen (7), and it has been suggested that changes in the EGF receptor might be involved in the toxicity of TCDD in neonatal mice (early eye opening, tooth eruption, and hair growth) (28), the effects of TCDD on differentiation of keratinocytes (29), and possibly the hepatocarcinogenic actions of TCDD (10). The present dose-response studies, using congenic female C57BL/6J mice differing only at the *Ah* locus, clearly show that the *Ah* locus mediates the effects of TCDD on the hepatic EGF receptor. The 10-fold increase in the ED_{50} for the effects of TCDD on EGF receptor binding in Ah$^{d/d}$ mice is similar to the increase in the ED_{50} for the induction of P4501A1 and its EROD activity

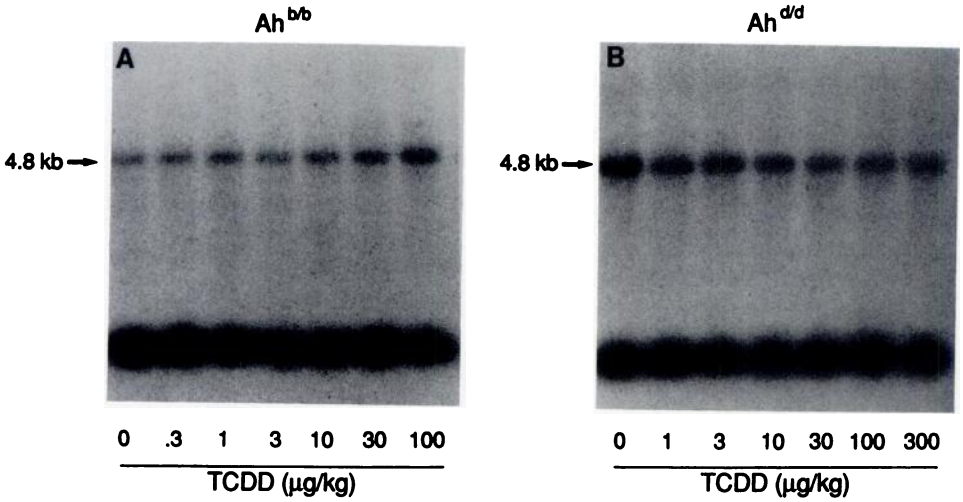

Fig. 5. Autoradiographs of Northern blots of TGF-α mRNA in livers of Ah$^{b/b}$ (A) and Ah$^{d/d}$ (B) mice after TCDD treatment. Blots were prepared as described in Fig. 4. Blots were then stripped and rehybridized with a rat ^{32}P-labeled TGF-α cDNA probe, as described in Materials and Methods. Each lane represents mRNA (6 μg) from a single animal. *Arrows*, the 4.8-kb mRNA transcript.

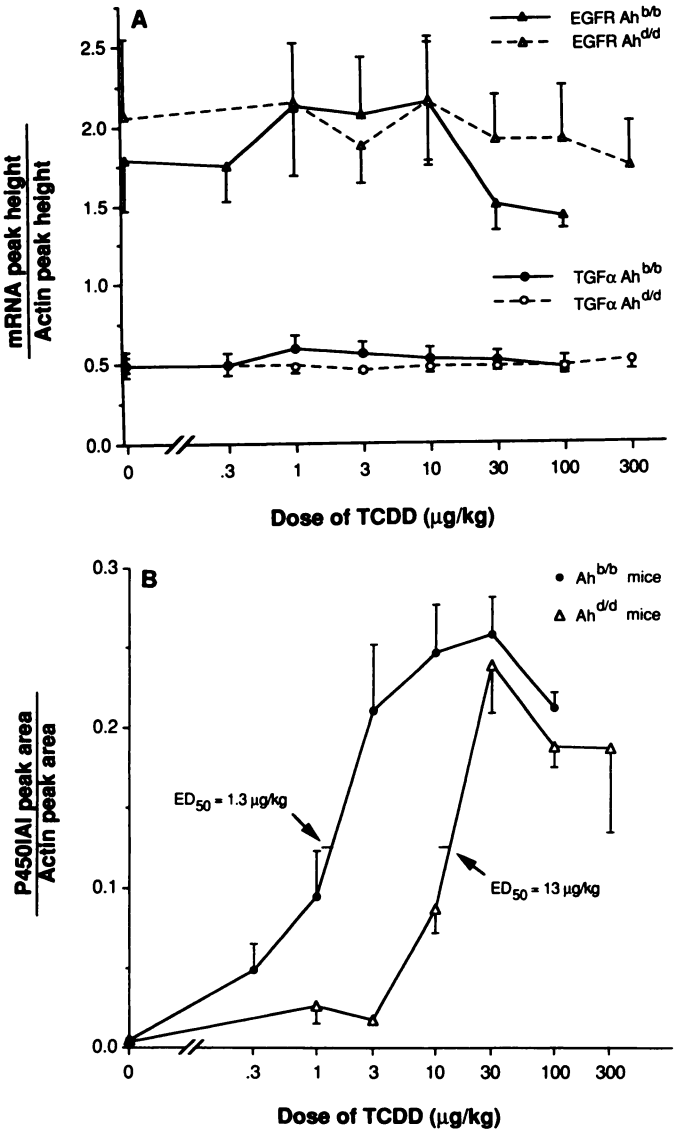

Fig. 6. Dose-response curves comparing the effects of TCDD on the hepatic content of the mRNAs for the EGF receptor (*EGFR*) and TGF-α (A) or P4501A1 (B) in $Ah^{b/b}$ versus $Ah^{d/d}$ mice, 7 days after treatment. Northern blots were prepared with 2 and 6 μg of hepatic mRNA from each animal and were hybridized as described in Materials and Methods. The same blots were hybridized with ^{32}P-labeled actin cDNA, stripped, and sequentially rehybridized with a ^{32}P-labeled EGF receptor antisense RNA riboprobe, a ^{32}P-labeled cDNA for rat TGF-α, and finally a ^{32}P-labeled cDNA for P4501A1. The blots were autoradiographed, and the major band for EGF receptor, TGF-α, or P4501A1 was scanned by laser densitometry. All values represent the mean ± standard error (peak height or peak area/corresponding value for actin mRNA) (n = 4). TCDD did not produce a statistically significant effect on hepatic EGF receptor mRNA or TGF-α mRNA, by analysis of variance ($p > 0.05$). TCDD significantly increased P4501A1 mRNA in $Ah^{b/b}$ mice at doses above 0.3 μg/kg and in $Ah^{d/d}$ mice at doses above 10 μg/kg ($p < 0.05$), using analysis of variance and Fisher's least significant difference test. The ED_{50} for this effect was significantly different in the two strains of mice ($p = 0.001$) (t tests).

(24), effects known to be mediated by the Ah receptor (1–3). Recent studies in our laboratory, using these congenic mice, have indicated that the *Ah* locus also mediates the effect of TCDD on the estrogen receptor (24). However, we could not show any influence of the *Ah* locus on TCDD-mediated decreases in glucocorticoid receptor binding, suggesting that an alternate mechanism may mediate the effects of TCDD on this receptor.

Scatchard analysis of EGF binding indicates that the decrease in EGF binding is due to a reduction in the number of receptor sites, rather than an altered affinity of the receptor, as previously reported by Madhukar *et al.* (6) and Osborne *et al.* (11) for liver (*in vivo*) and keratinocytes (*in vitro*). The mechanism responsible for the change in EGF receptor binding is not known. The TCDD-Ah receptor complex has been shown to induce P4501A1 by binding to a specific enhancer sequence in the upstream region of the *CYP1A1* gene, initiating transcription of the hepatic content of the mRNA for this protein (2, 3). It has been suggested that the Ah receptor-ligand complex may act in a similar manner to transcriptionally activate a battery of TCDD-responsive genes (1). However, the present study shows that TCDD does not affect the mRNA for the EGF receptor, suggesting that it does not act by influencing synthesis of the EGF receptor protein. Osborne *et al.* (11) also found that TCDD decreased EGF receptor binding in a human keratinocyte cell line but did not alter the amounts of EGF receptor mRNA. These results contrast with the effects of TCDD on the mRNA for P4501A1 in the present study. However, these studies do not show whether TCDD affects the level of the EGF receptor protein by an alternate mechanism.

The EGF receptor can also be down-regulated by binding of ligand followed by internalization and lysosomal degradation (30, 31). However, EGF is not synthesized in the liver (18, 26). Moreover, Madhukar *et al.* (28) reported that TCDD does not affect serum EGF levels in C57BL/6J mice or rats, under treatment conditions that decreased hepatic EGF receptor binding. TGF-α is an alternative ligand for the EGF receptor (8) that is synthesized in the liver and is proposed to be a physiological regulator of liver regeneration (9). The hepatic content of the mRNA for TGF-α is increased during liver regeneration. Therefore, it seemed possible that changes in EGF receptor number after TCDD treatment might also reflect changes in the synthesis of TGF-α. Although we did not measure the amount of TGF-α in the liver, we did not find any effect of TCDD on the hepatic content of the mRNA for TGF-α, indicating that synthesis of TGF-α is not affected by TCDD.

Changes in the phosphorylation of the EGF receptor are a possible alternative mechanism for regulation of the EGF receptor (32). The tumor promotor TPA directly activates protein kinase C (33), which phosphorylates the EGF receptor at threonine-654. This results in decreased binding capacity of the EGF receptor (25), a decrease in ligand-dependent autophosphorylation, and internalization of the receptor (34, 35). These effects occur within a few minutes after exposure to TPA (36), in contrast to the delayed effects of TCDD on EGF receptor binding. TCDD has been reported to increase protein kinases in liver, including protein kinase C (28, 37). However, these changes in kinase activities also occurred relatively long after TCDD exposure (12 to 24 hr), and their relationship to EGF receptor binding capacity is not known.

The EGF receptor could also be regulated by changes in membrane insertion or changes in degradation of the EGF receptor. EGF receptor binding can be down-modulated by changes in other growth factors, such as platelet-derived growth factor (38), interleukin-1, and tumor necrosis factor (39). Therefore, changes in any of a number of cellular growth factors

might conceivably mediate the effects of TCDD on the EGF receptor.

In conclusion, our results show that the effects of TCDD on the hepatic EGF receptor are mediated via the *Ah* locus in congenic strains of mice differing at this locus and are, therefore, mediated by the Ah receptor. However, unlike the effects of TCDD on P4501A1 mRNA, the effect on the EGF receptor was not a pretranslation effect on EGF receptor mRNA. Moreover, the effects of TCDD on the EGF receptor did not involve changes in the mRNA for TGF-α, an alternate ligand for the EGF receptor that is synthesized in the liver.

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