Tissue Distribution of the Receptor for 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Rat

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ABSTRACT

The tissue distribution of the receptor protein for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in adult male Sprague-Dawley rats was determined by isoelectric focusing in polyacrylamide gel of cytosol labeled with [3H]TCDD. In order that the TCDD receptor focuses as a single sharp peak at pl 5.2, partial proteolysis of the receptor must first be carried out. The radioactivity bound to this peak was completely displaced when labeling of the cytosol was carried out in the presence of 100-fold unlabeled 2,3,7,8-tetrachlorodibenzo-furan.

The highest concentration of the TCDD receptor was found in thymus (25.2 fmol/mg protein; n = 10) and lung cytosol (20.8 fmol/mg protein). No significant difference was seen between the concentration of the receptor in liver (13.9 fmol/mg protein) and kidney cytosol (12.5 fmol/mg protein). Low concentrations of the receptor were found in testis (3.5 fmol/mg protein), brain (2.8 fmol/mg protein), and skeletal muscle cytosol (0.3 fmol/mg protein). No receptor could be detected in the cytosol from pancreas, the adrenal glands, or ventral prostate.

Ventral prostate was found to contain a different binding species for [3H]TCDD than the receptor. This binder focused at pl 4.7. It was not affected by incubation with the same concentration of trypsin used to partially degrade the TCDD receptor. Neither could the prostatic TCDD-binding protein be saturated by incubation in the presence of 100-fold unlabeled 2,3,7,8-tetrachlorodibenzo-furan.

INTRODUCTION

TCDD has been shown to be a very potent inducer of AHH in several different mammalian systems (18—20, 22). Poland and Kende (23) demonstrated in mice that the response to treatment with TCDD with regard to AHH induction varied greatly between different strains. This variation was shown to be regulated by a single genetic locus, the Ah locus (16, 17, 27). It was postulated that the product of the Ah locus was a receptor for polycyclic aromatic hydrocarbons, and it was later shown that liver cytosol from responsive strains of mice or rats could bind TCDD with high affinity and low capacity and that this binding was stereospecific for those polycyclic aromatic hydrocarbons capable of inducing AHH (21). This high-affinity binding only occurs in the liver cytosol from responsive rats or mice and cannot be demonstrated in cytosol from nonresponsive animals (21, 24). With the help of isoelectric focusing in polyacrylamide gel, a single binding species for TCDD could be demonstrated in liver cytosol (3). Scatchard plots for the binding of TCDD measured by using this method gave a Kd of 0.6 nm. Binding of TCDD to the peak focusing at pl 5.2 was competed for by 3-methylcholanthrene, β-naphthoflavone, and TCDBF, all inducers of AHH, but not by phenobarbital or pregnenolone-16α-carbonitrile, inducers of other forms of cytochrome P-450.

TCDD has been shown to induce AHH in lung, prostate, and testis (9, 10). Treatment with TCDD results in thymus involution (5, 12) and an immunodepression (5). Morphological changes have been seen in the kidney following treatment with TCDD (6). Thus, TCDD has a widespread effect in various tissues, and therefore the distribution of the TCDD receptor has been studied by quantitation by isoelectric focusing in polyacrylamide gel.

MATERIALS AND METHODS

Eight-week-old male Sprague-Dawley rats were anaesthetized with ether and perfused via the left ventricle with 60 ml of ice-cold ETG buffer. The rats were then perfused via the inferior vena cava with 120 ml of ice-cold ETG buffer. During this perfusion, the liver was gently massaged to ensure an even perfusion and the removal of as much blood as possible. Finally, the inferior vena cava was cut above the liver and clamped. The lungs were then perfused via the right atrium with 60 ml of ice-cold ETG buffer.

Following perfusion, the liver, thymus, lungs, kidneys, adrenals, testes, pancreas, skeletal muscle, and brain were removed onto ice. The kidneys were separated from their capsule before dissection. The pancreas was carefully cleaned of all surrounding fat. The skeletal muscle was taken from the right quadriceps muscle and carefully dissected free of all fat. The brain consisted of cerebrum, cerebellum, and medulla oblongata. All further work was carried out at 2 to 4°C.

Preparation of Cytosol. The liver was homogenized in 24 ml ETG buffer, and the thymus, lungs, kidneys, testes, pancreas, and brain were each homogenized in 10 ml buffer with a glass Potter-Elvehjem homogenizer fitted with a Teflon pestle. Muscle was homogenized in 10 ml ETG buffer with an Ultra-Turrax tissue homogenizer. The adrenals were homogenized in an all-glass hand-driven Potter-Elvehjem homogenizer in 1 ml of buffer.

The adrenal homogenate was centrifuged directly at 150,000 x g for 45 min. The other homogenates were first centrifuged at 20,000 x g for 15 min, and the resulting supernatant was then centrifuged at 150,000 x g. After centrifugation, the floating lipid layer was carefully removed by aspiration, and the resulting supernatant was used as cytosol after dilution to 3.5
**Tissue Distribution of TCDD Receptor**

**In Vitro Labeling of the Cytosol.** [1,6-3H]TCDD (specific activity, 36.6 Ci/mmol) was a kind gift from Dr. Alan Poland (McArdle Laboratory for Cancer Research, Madison, Wis.) and was stored in the dark at 2 to 4°C in toluene:ethanol (4:1). Prior to labeling of the cytosol, an aliquot of the [3H]TCDD solution was evaporated to dryness under a stream of nitrogen and redissolved in dioxane at a concentration of 300 nm. To each ml of cytosol, 5 μl of the dioxane solution was added, yielding a concentration of 1.5 nm (for the saturating concentration, see Ref. 3), and the cytosol was incubated at 0°C for 2 hr.

**Limited Proteolysis of the Receptor.** After labeling of the receptor with [3H]TCDD, the cytosol was incubated with trypsin prior to isoelectric focusing (3). Trypsin:TPCK (269 units/mg; Worthington Biochemical Corp., Freehold, N.J.) was dissolved in water, and 10 μl of the solution were added per ml of cytosol. Optimal recovery of the receptor from incubations with lung or brain cytosol was achieved after incubation of the cytosol with 2 μg trypsin per A280 - 310 for 30 min at 10°C. Optimal recovery of the receptor from incubations with the other cytosols was achieved following incubation of the cytosol with 0.5 μg trypsin per A280 - 310 for 30 min at 10°C.

**Isoelectric Focusing in Polyacrylamide Gel.** Free TCDD was removed from the trypsinized labeled cytosol by treating 0.5 ml of the incubate with 150 μl of a dextran-coated charcoal suspension [3.8% (w/v) activated charcoal and 0.38% (w/v) Dextran T-500 (Pharmacia, Uppsala, Sweden) in ETG buffer] for 10 min at 0°C. The mixture was then centrifuged at 3,250 g for 10 min, and 300 μl of the resulting supernatant was analyzed by isoelectric focusing in polyacrylamide gel as previously described (3). Nine samples could be analyzed simultaneously, and focusing was carried out for 2 hr. After focusing, the pH was measured, and the gel strip corresponding to each sample was cut into 3-mm fractions. The radioactivity was measured. pH gradient (upper right box) is uniform for all the samples. dpm/fr, dpm/mg.

**RESULTS**

As previously described (3), a specific binding species for TCDD can be demonstrated in rat liver cytosol by isoelectric focusing in polyacrylamide gel. In its native form, this protein focuses as a broad peak with a pI of approximately 6.2. If, however, the liver cytosol is incubated with trypsin after labeling of the receptor with [3H]TCDD, the radioactivity focuses as a single sharp peak with pI 5.2. Furthermore, the recovery of radioactivity following trypsinization is greater than that from the untreated cytosol. Thus, limited proteolysis of the TCDD receptor results in a fragment that focuses in a reproducible manner and increases the recovery of bound radioactivity. For liver cytosol, the optimal trypsin concentration was found to be 0.5 μg/A280 - 310 (3). This concentration of trypsin resulted in the highest recovery of bound radioactivity and was also found to be optimal for incubations with thymus, kidney, testis, pancreas, muscle, or adrenal cytosol. Incubations with lung or brain cytosol were found to require a higher concentration of trypsin, 2 μg/A280 - 310, to give optimal recovery of bound radioactivity in the peak at pI 5.2.

Chart 1 shows the isoelectric focusing pattern for incubations with the different cytosols from one rat. In addition to the liver, the thymus, lungs, and kidneys contained a significant amount of TCDD receptor in the cytosol as seen by the peaks at pI 5.2. Cytosols from testis, brain, and muscle also contained TCDD receptor, but the peaks were much smaller. Cytosol from pancreas or adrenal glands did not contain any bound radioactivity when analyzed by isoelectric focusing. In all cases in which the peak at pI 5.2 occurred, the radioactivity could be completely displaced by incubation of the cytosol with [3H]-TCDD in the presence of a 100-fold concentration of unlabeled TCDBF. Trypsinization of the receptor in brain cytosol was achieved after incubation of the cytosol with 2 μg trypsin per A280 - 310 for 30 min at 10°C. Optimal recovery of the receptor from incubations with the other cytosols was achieved following incubation of the cytosol with 0.5 μg trypsin per A280 - 310 for 30 min at 10°C.
frequently inadequate, in which case the receptor focused at pl 6.2. However, the radioactivity in this peak could also be displaced by TCDBF, and therefore the TCDD receptor content in brain cytosol was quantitated from both peaks.

The amount of receptor in the different cytosols can be seen in Table 1. The values given in Table 1 represent the concentration of free receptor sites in the cytosol as calculated from the radioactivity focusing in the peak at pl 5.2. This radioactivity was shown to be specifically bound by competition with unlabeled TCDBF. Part of the bound radioactivity is lost during the analysis owing to the time required for completed focusing. Lung cytosol and thymus cytosol contained significantly more TCDD receptor ($p < 0.05$; Student's $t$ test) than did liver cytosol. The concentration of TCDD receptor in kidney cytosol was not significantly different from the concentration in liver cytosol. Brain, testis, and muscle cytosol contained significantly less receptor ($p < 0.001$) than did liver cytosol. No receptor could be detected in adrenal or pancreas cytosol. The minimum detection level was approximately 0.3 fmol per ml cytosol (assuming one ligand-binding site per receptor molecule).

Ventral prostate cytosol (Chart 2) was found to contain a TCDD-binding species distinct from the TCDD receptor found in the other organs. This binding species focused at a pl of about 4.7 and was not affected by trypsinization. The prostas TCDD binder could not be saturated by the addition of a 100-fold concentration of unlabeled TCDBF.

**DISCUSSION**

Poland et al. (21) showed that there occurred a stereospecific high-affinity binding of $[^3H]$TCDD in rat and mouse liver cytosol. This high-affinity binding could only be found in liver cytosol from strains of mice that are responsive to TCDD with respect to AHH induction, such as the C57BL/6J strain (21, 24). Nonresponsive strains, such as the DBA/2J strain, did not bind TCDD. Analysis of rat liver cytosol labeled with $[^3H]$TCDD, by isoelectric focusing, demonstrated the occurrence of a distinct binding species for $[^3H]$TCDD (3). This binder was stereospecific for those polycyclic aromatic hydrocarbons capable of inducing AHH but did not bind other inducers of microsomal monooxygenases, such as phenobarbital. Scatchard plots for this binding showed a high affinity, $K_d = 0.6$ nm, and a low capacity, 30 fmol/mg protein.

After binding of $[^3H]$TCDD to the high-affinity binder in liver cytosol, the complex can bind to DNA. Before the binding of TCDD to the high-affinity binder, the binder does not interact with DNA. Trypsinization of the TCDD:binder complex destroys its DNA-binding capacity without affecting the binding of TCDD. This is analogous to the behavior of other intracellular receptor proteins, such as the glucocorticoid receptor (4, 29) or the progesterone receptor (2, 25, 26). Furthermore, it has previously been demonstrated that the induction of AHH by polycyclic aromatic hydrocarbons (13, 15), in particular by TCDD (8), can be blocked by actinomycin D or by cycloheximide administered simultaneously. However, if these agents are administered after the hydrocarbon, they are not effective. Thus, the induction of AHH requires an initial transcription followed by translation. Haugen et al. (7) and Kitchin and Woods (8) have shown that the induction of AHH in the liver by TCDD involves de novo synthesis of cytochrome P-450.

Altogether, these data indicate that TCDD and other polycyclic aromatic hydrocarbons exert their effect via an intracellular receptor and that this receptor, after binding of its ligand, interacts with the genome. The binding of TCDD to the receptor and its interaction with DNA results in specific activation of a number of genes, among them the gene for AHH. This receptor has previously been described as an important product of the Ah locus (1, 24, 28). The binding species for TCDD demonstrated in rat liver cytosol by isoelectric focusing demonstrates all of the characteristics for an intracellular receptor protein.

**Table 1**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TCDD receptor concentration (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>13.9 ± 4.3²</td>
</tr>
<tr>
<td>Thymus</td>
<td>25.2 ± 11.0</td>
</tr>
<tr>
<td>Lung</td>
<td>20.8 ± 5.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>12.5 ± 5.0</td>
</tr>
<tr>
<td>Testis</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td>Brain</td>
<td>2.8 ± 1.8</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0</td>
</tr>
</tbody>
</table>

² Assuming one ligand-binding site per receptor molecule.

3 J. Carlstedt-Duke, U-B. Harnemo, B. Hogberg, and J-A. Gustafsson. Physical characterisation of the hepatic receptor protein for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and its interaction with DNA. Submitted to Journal of Biological Chemistry.
and is therefore described as the TCDD receptor. A similar electrophoresing pattern was seen in the cytosol from extrahepatic tissues. There occurred a peak of radioactivity focusing at pI 5.2 following the incubation of the cytosol with [3H]TCDD and subsequent treatment with trypsin. As the peak at pI 5.2 could always be saturated with 100-fold unlabeled TCDBF, the binder in the extrahepatic tissues was a low-capacity binder and was designated the putative TCDD receptor even in these tissues.

AHH can be induced in extrahepatic tissues, in addition to the liver, by polycyclic aromatic hydrocarbons. Nebert and Gelboin (13) found inducible AHH activity in fetal hamster cells derived from the liver, lung, small intestine, limb, or brain. They also demonstrated that AHH can be induced in vivo in the rat, monkey, and hamster in the liver, lung, gastrointestinal tract, and kidney (14). High levels of TCDD receptor were found in this study in those tissues reported to contain inducible AHH activity (gastrointestinal tract not investigated).

In addition to the induction of AHH and gluturonyltransferase by TCDD in the kidney, ultrastructural effects can also be seen (6). These effects were only seen in the renal proximal tubule cells of the straight (S3) segments. The ultrastructural effects involved the proliferation of smooth endoplasmic reticum, and this occurred only in the cells that demonstrated enzyme induction.

The thymus was found to contain the highest level of TCDD receptor of the tissues analyzed in this study. Faith and Moore (5) have reported the impairment of thymus-dependent immune systems in F344 rats following exposure to TCDD. TCDD suppressed cell-mediated immune function, specifically ‘helper’ T-cell function, without affecting humoral immune function. Furthermore, TCDD resulted in a thymus involution in these rats. McConnell et al. (12) have also shown thymus involution following the exposure of rhesus monkeys to a single dose of TCDD.

The TCDD-binding protein found in the prostate did not demonstrate the characteristics of a receptor protein (high affinity, low capacity). This binder does not interact with DNA: cellulose. However, the occurrence of this binding species is very interesting in view of the report of a 150-fold induction of AHH in the rat prostate by Lee and Dixon (9). This is a much higher level of induction than that in the liver. In addition, the persistence of the AHH induction is much longer in the prostate (9, 10). They also reported a slight induction of AHH activity in the testis, which corresponds to the low level of TCDD receptor found in the testis.

The significance of the prostatic TCDD:binder is not known. This protein occurs in a much larger concentration than that of the TCDD receptor in other tissues. In addition, it has a much larger binding capacity than the TCDD receptor and, it would appear, a relatively high affinity for TCDD. It is possible that this binder masks the occurrence of TCDD receptor in the prostate since it focuses very close to the receptor and occurs in such large amounts. It is probable that the persistent high induction of AHH in the prostate is due to the concentration of TCDD in this organ, bound to the prostatic TCDD-binding protein.

In conclusion, the tissue distribution of the TCDD receptor is very similar to the reported distribution of the effects of TCDD, especially with regard to the induction of AHH activity. One exception to this is the induction of AHH activity in the prostate where another TCDD-binding protein is found.

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REFERENCES


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