Uptake and Specific Binding of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Olfactory Mucosa of Mice and Rats

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ABSTRACT

Whole-body autoradiography of mice and rats after i.v. administration of 2,3,7,8-4Htetrachlorodibenzo-p-dioxin (4HjTCDD) showed a selective localization of radioactivity in the liver and nasal olfactory mucosa. In microautoradiograms of solvent extracted sections of the skulls of mice given injections of 4HjTCDD, no radioactivity was observed in the olfactory mucosa, suggesting that TCDD is not covalently bound in this tissue.

The amount of specific 4HjTCDD binding sites in cytosol from the ethmoturbinate of rats (33 fmol/mg cytosolic proteins) was comparable to that of the liver cytosol as estimated by electrophoresis in polyacrylamide gradient concentration gel, and therefore probably too low to explain the retention of radioactivity in the olfactory mucosa. The specific TCDD binding species in the mucosa of the ethmoturbinate exhibited a similar binding affinity for 4HjTCDD, ligand specificity, and molecular weight as the TCDD receptor from rat liver. The 7-ethoxyresorufin O-deethylase activity of the mucosa of the ethmoturbinate was induced less than twice by administration of the TCDD receptor ligand β-naphthoflavone (5,6-benzoflavone) 40 h before killing. By administration of β-naphthoflavone (5,6-benzoflavone) 16 h before killing, mRNA coding for cytochrome P-450d but not for cytochrome P-450c was induced to detectable levels in the mucosa of the ethmoturbinal tissue of the rat.

The basal activity of 7-ethoxyresorufin O-deethylase of the mucosa of the ethmoturbinal tissue of the rat was similar to the corresponding activity of the liver. This basal metabolic activity of the ethmoturbinal tissue was only marginally inhibited by antibodies raised against β-naphthoflavone (5,6-benzoflavone) induced hepatic cytochrome P-450s. Thus, enzymes other than cytochrome P-450c may possibly account for a part of the basal 7-ethoxyresorufin O-deethylase activity in the rodent olfactory mucosa.

INTRODUCTION

TCDD is one of the most toxic low-molecular compounds known. Similar to certain structurally related chlorinated compounds, e.g., biphenyls, dibenzo furans, and dibenzodioxins, TCDD exerts characteristic effects in animals (2). In most species these include a wasting syndrome, lymphoid tissue involution, teratogenesis, and induction of certain enzymatic activities, e.g., aryl hydrocarbon hydroxylase activity. In some species TCDD is also hepatotoxic, causes hyperkeratosis, and chloracne (2). A soluble receptor protein, the TCDD receptor, that binds TCDD, BNF, and structurally related polycyclic aromatic hydrocarbons (3, 4) that are inducers of the same activities, e.g., 7-ethoxyresorufin O-deethylase activity of the mucosa of the ethmoturbinal tissue of the rat. The amount of 7-ethoxyresorufin O-deethylase activity in the rodent olfactory mucosa.

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2 The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; BNF, β-naphthoflavone (5,6-benzoflavone); cytochrome P-450d, the major form of cytochrome P-450 isolated from liver microsomes of BNF-treated rats; cytochrome P-450c, the major form of cytochrome P-450 isolated from liver microsomes of isosafrole-treated rats; cDNA, complementary DNA.

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Since TCDD and 2,3,7,8-tetrachlorodibenzo-p-dioxin are extremely toxic compounds (2), we used the special handling procedures outlined by Poland and Glover (3), and the contaminated disposable materials were sent away for high-temperature incineration.

**Safety Precautions**

**In Vitro Studies**

**Autoradiography**. The radiolabeled TCDD was injected i.v. (in a tail vein) or i.m. (in the hind leg) in mice or rats. The animals were killed at post-injection times ranging from 5 min to 61 days.

After killing, the animals were immersed in an aqueous gel of carboxymethyl cellulose and frozen in n-hexane cooled with solid CO2 (−78°C). The frozen animals were sectioned for autoradiography as described by Ullberg (8). Sagittal whole-body or transversal sections of the heads (20 µm thick) were taken on a PMV cryostat microtome (LKB, Stockholm, Sweden) at −20°C. The tape-fastened sections were freeze-dried and apposed to X-ray film.

**Microautoradiography.** Mice were given an i.v. injection of [³H]TCDD and killed after 24 h. Their entire nasal regions were removed and fixed in 4% (w/v) formaldehyde in sodium phosphate buffer (pH 7.0). After fixation, the tissues were decalcified in 5.5% (w/v) EDTA for about 1 week. The tissues were then dehydrated in an ethanol series before being embedded in Historesin. Sections 2-µm thick were cut and mounted on glass slides. The sections were transferred twice for 5 min in xylene and then hydrated in a series of ethanol before the slides were dipped in NTB-2 liquid film emulsion and stored up to 10 months at 4°C in the dark. The film was then developed and the adhering sections were stained with hematoxylin-eosin.

**In Vitro Studies**

**Nasal Tissue Preparation for Enzyme Assay.** Rats were decapitated and the skin was removed from the top of the skull and nose. The skull was split open longitudinally with a scalpel. The nasal tissues were removed and placed in 0.1 M Tris-HCl buffer (pH 7.4) containing 1.15% (w/v) formaldehyde in sodium phosphate buffer (pH 7.0). At fixation, the samples were decalcified in 5.5% (w/v) EDTA for about 1 week. The tissues were then dehydrated in an ethanol series before being embedded in Historesin. Sections 2-µm thick were cut and mounted on glass slides. The sections were transferred twice for 5 min in xylene and then hydrated in a series of ethanol before the slides were dipped in NTB-2 liquid film emulsion and stored up to 10 months at 4°C in the dark. The film was then developed and the adhering sections were stained with hematoxylin-eosin.

**Liver Preparation for Enzyme Assay.** The livers of rats were excised and immediately immersed in chilled 0.25 M sucrose for washing. Subsequent steps were performed at 4°C. Two livers were homogenized in 2 vol of 0.25 M sucrose with a Potter-Elvehjem glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 100,000 x g for 60 min. The pellet was resuspended in 0.1 M potassium pyrophosphate buffer (pH 7.4) containing 1 mM EDTA and diluted with the buffer used for 7-ethoxyresorufin O-deethylase activity determination.

**7-Ethoxyresorufin O-Deethylase Activity Assay.** Freshly prepared particulate fractions were always used in the assay. The O-deethylation of 7-ethoxyresorufin was followed essentially as described by Burke and Mayer (9) with a Shimadzu RF-510 spectrophotofluorometer (λex = 550 nm; λem = 585 nm). Duplicate incubations containing 0.1 M Tris-HCl (pH 7.8) were prepared in a total volume of 1 ml directly in the fluorometric cuvet. Twenty µl of the particulate fractions were added (except for BNIF induced liver microsomes where 2 µl were added) to the cuvet. The concentration of protein in the particulate fractions ranged between 5 and 20 mg/ml; 10 nmol of substrate were added in 10 µl of dimethyl sulfoxide. After a stable baseline had been obtained at 37°C, the reaction was started by the addition of 5 µl 50 mM NADPH. Calibration was achieved by addition of 5 µl 5 µM rhodamine (under these analytical conditions corresponding to 75 pmol of 7-hydroxyresorufin).

**Antibody Inhibition of Enzymatic Activity.** The antibodies (0.5 mg) were incubated in duplicate for 30 min at 25°C with ethmoturbinal particulate fractions (0.15 mg protein) in a total volume of 100 µl buffer prior to determination of 7-ethoxyresorufin O-deethylation.

**Preparation of Nasal Homogenate for Receptor Assay.** The ethmoturbinal tissue was obtained in the same way as for enzyme assays, blotted on paper to remove blood, and immersed in ice-cold buffer, consisting of 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 10% (w/v) glycerol, and 2 mM 2-hydroxyethylmercaptopane. This buffer was used in all subsequent steps of the receptor assay. The ethmoturbinals from five rats were pooled (total weight, 0.7–1 g) and homogenized for 2 x 5 s with an Ultraturrax in 3 vol of buffer.

**Preparation of Cytosol.** The homogenate was centrifuged at 140,000 x g for 45 min in a fixed angle 50 rotor in a Beckman L-8 M ultracentrifuge. The clear supernatant was used as cytosol after dilution to 1.5 x 10^10,000 nm/ml (corresponding to 2–4 mg protein/ml).

**Proteina Determination.** Protein concentration of microsome suspensions and cytosols was always determined according to Lowry et al. (10) with bovine serum albumin as the standard.

**In Vitro Labeling of Cytosol.** To each 0.5-ml aliquot of cytosol in borosilicate glass tubes, 5 µl of a 300 nm [³H]TCDD solution, or dilutions thereof were added. Dimethyl sulfoxide was also used as solvent for unlabeled competitors. When necessary, dimethyl sulfoxide was added to keep the total solvent concentration constant in all incubations of one experiment. [³H]TCDD was always added as the final step. The amount of nonspecific binding was monitored in all experiments with an incubation containing 1.5 nm [³H]TCDD and 300 nm 2,3,7,8-tetrachlorodibenzo-p-dioxin.

To estimate their binding affinity for the TCDD receptor, ligands were incubated in duplicate with cytosol at 150 nm concentration in the presence of 1.5 nm [³H]TCDD. In such experiments an incubation with 1.5 nm [³H]TCDD without competitor was used to determine total binding, which was utilized for calculation of maximum specific binding.

In order to estimate the number of specific TCDD binding sites by saturation analysis, cytosol was incubated with various amounts of [³H]TCDD to yield final concentrations ranging from 0.15–3.0 nM.

**Assay of Specific Binding in [³H]TCDD-labeled Cytosol.** After 2 h, limited proteolysis (if not otherwise indicated) and dextran-coated charcoal treatment of the cytosol were carried out using the same conditions as in the electrofocusing assay for the TCDD receptor described earlier (4). The resulting supernatants were analyzed by polyacrylamide concentration gradient gel electrophoresis which will be described in detail elsewhere; only a brief description is given here. Polyacrylamide concentration gradient gels (2.5–20%; 110 x 245 mm) were prepared and run under nondenaturing conditions in a LKB Multiphor apparatus. The samples and molecular weight marker proteins were applied in a similar manner as used for electrofocusing (4), and the running buffer consisted of 90 mM Tris-HCl, pH 8.35 (at 25°C), 80 mM boric acid, and 2.5 mM EDTA. The electrophoresis was carried out overnight (18 h) at 167 V and 4°C. In front of each sample frame, 6-cm long and 2-cm wide sections were cut from the gel and sliced into ten 4-mm sections (for molecular weight estimations, 2-mm sections)

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with a pair of scissors. The slices were placed in plastic scintillation vials, treated, and counted as described for samples collected for electrophoresis analyses (4). The area afront of the application of the marker proteins was cut out and stained with Coomassie brilliant blue after complete electrophoresis.

Molecular Weight Estimation and Quantification of Specific [3H]TCDD Binding. The relative migration distances of the stained marker proteins were plotted against the log of their molecular weights (as given by the supplier). The molecular weight of the [3H]TCDD binding species was interpolated from the plot using linear regression. The total binding of [3H]TCDD in the cytosol was quantitated as the area below the radioactivity peaks corresponding to the molecular weight of the TCDD receptor observed with this method, following electrophoresis of the incubation mixtures. The nonspecific binding was calculated and subtracted from the total binding in each incubation to obtain the specific binding, as described by Chamness and McGuire (11) for analysis of steroid hormone receptor-binding data.

Plasmids. Specific cDNA recognizing the 3’ noncoding region of cytochrome P-450c mRNA (bases 1506 to 1771; 262 base pairs) (12), was evident that the overall distribution pattern (Fig. 1) was disclosed no major differences in the studied mouse strains. It was observed in the NMRI strains, the distribution of radioactivity in the tissues given injections as above and killed after 1 and 24 h and 4 days. No significant sex differences in the tissue distribution of radioactivity were noted in the C57BL strain mice were given injections as above and killed after 20 min, 24 h, and 4 days. No significant sex differences in the tissue distribution of radioactivity were noted in the C57BL strain (data not shown).

Up to 24 h after injection, the radioactivity was irregularly localized in the liver, with the most conspicuous labeling around the central veins. Two months after injection, a low amount of radioactivity still persisted in this tissue. The contents of the gallbladder were labeled 4 and 24 h after the C57BL, CBA, and NMRI mice were given injections. In the mice killed 4 h-15 days after the injections there was also a labeling of the intestinal contents. Four h after the injections, the intensity of the labeling of the brown fat was about equal to that of the liver (data not shown). The radioactivity in this tissue gradually decreased; a low level was still present 1 month after the injection.

Up to 1 h after mice had received injections of [14C]TCDD, the level of radioactivity in the adrenal cortex was high (Fig. 2) and about equal to that of the liver, but at longer postinjection times the intensity of the labeling of the adrenals was low as compared to that of the liver. Except for the adrenal cortex, no site of accumulation of radioactivity was observed in the endocrine system. The labeling of the thymus (Fig. 1), lymph nodes, bone marrow, and prostate was also negligible at all postinjection times. In agreement with earlier studies (6, 7), the level of radioactivity in the kidneys and spleen was also low (Fig. 2).

In order to study the placental transfer of TCDD, three pregnant C57BL mice were given i.v. injections of [14C]TCDD (420 nmol/kg; 1.0 £Ci/40 M) and killed after 1 and 24 h and 4 days; all were killed on the 17th day of gestation (the day after mating was designated as day 0 of gestation). In the pregnant mice, the fetal concentration of radioactivity was considerably lower than that of the dam at the three time intervals studied. A distinct uptake of radioactivity was, however, observed in the nasal mucosa and liver of the fetus (Fig. 3).

 Autoradiography of sagittal sections of C57BL, CBA, and NMRI mice after they had received injections of [14C]TCDD (420 nmol/kg; 1.0 £Ci/40 M) demonstrated a selective uptake of radioactivity in the nasal mucosa that was less accentuated in the C57BL mouse. Within the nasal region, the radioactivity was preferentially localized in the mucosa of the ethmoturbinate, but marked labeling also occurred in the mucosa of the posterior part of the septum and the lateral cavity walls. The radioactivity at these sites appeared at 4 h and became further accentuated at 24 h and later. A low amount of radioactivity still persisted here 1 month after injection. In contrast, the other sites of the respiratory system (the tracheobronchial mucosa and lung) consistently showed low amounts of radioactivity in these three mouse strains.

Since a pronounced accumulation of radioactivity was found in the nasal mucosa of mice after administration of [14C]TCDD, an experiment was carried out in order to study the uptake of radioactivity in this tissue in detail. One male mouse each (21 g) of the C57BL/6J, DBA/2J, and NMRI strains, respectively, was given an injection i.v. of [3H]TCDD (60 nmol/kg; 36 £Ci/25 M). After 24 h the mice were killed, and their heads and pieces of their livers were embedded and subjected to autoradiography. Transversal sections of the heads of these mice exhibited a marked localization of radioactivity in the mucosa of the ethmoturbinate, i.e., the olfactory mucosa (Fig. 4, A and B). No differences in the level of radioactivity in the olfactory mucosa between the three strains could be observed. In the anterior portion of the septa and lateral cavity walls, naso- and maxilloturbinates and in the vomeronasal organ the level of radioactivity was low. The level of radioactivity in the nasal tissue of the mice of the C57BL/6J strain was ~50% higher than in the livers of the mice of the DBA/2J strain, as judged by autoradiography (Fig. 4, C and D) and liquid scintillation counting.

RESULTS

In Vivo Studies

 Autoradiography. For whole-body autoradiography, one series of male C57BL mice (21 g) was given injections i.v. of [14C]TCDD (420 nmol/kg; 1 £Ci/40 M) and killed after 5, 20, and 60 min, 4 and 24 h, and 4, 15, 30, and 60 days. In a second series, two groups of male CBA and NMRI mice (21 g) were given injections as above and killed after 1 and 24 h and 4 days. With the exception that the labeling of the liver was considerably more intense in the C57BL strain than in the CBA and NMRI strains, the distribution of radioactivity in the tissues disclosed no major differences in the studied mouse strains. It was evident that the overall distribution pattern (Fig. 1) was dominated by a prominent accumulation of radioactivity in the liver. A selective uptake of radioactivity was also observed in the mucosa of the ethmoturbinates. In addition, female C57BL mice were given injections as above and killed after 20 min, 24 h, and 4 days. No significant sex differences in the tissue distribution of radioactivity were noted in the C57BL strain (data not shown).
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Fig. 1. Autoradiogram showing the distribution of radioactivity 24 h after an i.v. injection of [14C]TCDD in a male CBA mouse. There is a high level of radioactivity in the mucosa of the ethmoturbinates, the fat, and in the liver, whereas the level of radioactivity in other tissues, e.g., the thymus, is low.

Fig. 2. Detail of an autoradiogram showing the distribution of radioactivity 1 h after an i.v. injection of [14C]TCDD in a female C57BL mouse. There is a high level of radioactivity in the adrenal cortex and in the liver whereas the level of radioactivity in the kidney and spleen is low.

To establish if TCDD also is accumulated in the nasal tissues of rats, two male juvenile Sprague-Dawley rats (70 g) were given injections of [14C]TCDD, one i.m. (190 nmol/kg; 1.5 µCi/100 µl) and the other i.v. (325 nmol/kg; 2.6 µCi/100 µl). After killing, their heads were subjected to autoradiography. Transversal sections of their heads displayed a low but discernible uptake of radioactivity in the mucosa of the ethmoid region (data not shown).

Microautoradiography. In order to investigate if TCDD was firmly bound in any specific cell type in the olfactory mucosa microautoradiography of skulls from C57BL/6J and DBA/2J mice was performed as described in “Materials and Methods” 24 h after an i.v. injection of [3H]TCDD (60 nmol/kg; 36 µCi/25 µl). Since the sections were extracted with organic solvents, these autoradiograms will disclose only the radioactivity which is firmly (probably covalently) bound in the tissues. However, no radioactivity above the background could be observed in the olfactory mucosa within the exposure times used (up to 10 months) (data not shown).

In Vitro Studies

Assays of Specific [3H]TCDD Binding. The amount of specific [3H]TCDD binding in the ethmoturbinates of the rat was estimated in order to investigate if the presence of high levels of TCDD receptor could account for the apparently noncovalent binding of radiolabeled TCDD in this tissue. Due to the difficulty in preparing the mucosa of the ethmoturbinates completely free of blood, it was not possible to quantify the specific [3H]TCDD binding by saturation analysis using electrofocusing in polyacrylamide gel. A nonspecific [3H]TCDD binding species with an isoelectric point near that of the TCDD receptor is present in serum (19) and disturbs the assay when the cytosol is contaminated with blood. In this investigation we have instead applied polyacrylamide concentration gradient gel electrophoresis for the assay of specific [3H]TCDD binding sites. Polyacrylamide concentration gradient gel electrophoresis separates macromolecules according to their molecular weight rather than their charge. With this method linear Scatchard plots were obtained for specific [3H]TCDD binding in cytosol from the ethmoturbinates, with maximum numbers of specific [3H]TCDD binding sites (Bmax) ranging between 25–37 fmol/mg cytosolic protein, and apparent dissociation constants of the binding (Kd) ranging between 0.2 and 2.4 nM. A plot of the results from a typical experiment is shown in Fig. 5 with a Bmax ~33 fmol/mg cytosolic protein, and a Kd of ~0.5 nM for the specific [3H]TCDD binding. The level of specific [3H]TCDD and apparent dissociation constants of the binding in the ethmoturbinates appeared similar to or somewhat lower than the corresponding values in liver cytosol (Bmax = 36 fmol/mg cy-
Fig. 3. A, detail of an autoradiogram showing the distribution of radioactivity 4 days after an i.v. injection of [3H]TCDD in a pregnant C57BL mouse. There is a selective uptake of radioactivity in the mucosa of the fetal ethmoturbinates whereas the radioactivity in the fetal brain is low. B, corresponding hematoxylin-eosin stained section.

Fig. 4. Autoradiograms showing the distribution of radioactivity in excised heads (transversal sections) and pieces of livers 24 h after i.v. injections of [3H]TCDD in a female DBA/2J mouse (A and C) and a male C57BL/6J mouse (B and D). In A and B there are high and selective uptakes of radioactivity in the mucosa of the ethmoturbinates whereas the level of radioactivity in the rest of the head is low. The distribution of radioactivity in the livers (C and D) has a mottled appearance, probably due to a centriflobular uptake of radioactivity.

tosomal protein; $K_d = 1.1 \text{ nm}$), as judged by the same method. When [3H]TCDD-labeled ethmoturbinal cytosol was electrophoresed in polyacrylamide concentration gradient gels the native form of the specific [3H]TCDD binding species did not migrate as a distinct peak (Fig. 6A), but exhibited molecular weights ranging from 220,000–340,000. On the other hand, when the ethmoturbinal cytosol was subjected to limited proteolysis with trypsin a distinct peak (Fig. 6B) was observed with a molecular weight of $245,000 \pm 11,000$ (SD) ($n = 3$). Therefore, cytosols were routinely trypsinized for saturation analysis and study of ligand binding specificity. The molecular weight observed under similar conditions for the native and the trypsinized form of the specific [3H]TCDD binder in the liver is $316,000 \pm 27,000$ and $246,000 \pm 12,000$, respectively. The rank order of relative affinities of some well known TCDD receptor ligands for the specific [3H]TCDD binding
The molecular weights in this experiment were estimated as A. 344,000 and B, 232,000.

7-Ethoxyresorufin O-Deethylase Activity Assay. In the rat liver TCDD and certain TCDD receptor-binding polycyclic aromatic hydrocarbons (i.e., BNF and 3-methylcholanthrene) induce at least two distinct forms of cytochrome P-450 (c and d) (20). Corresponding forms of hepatic cytochrome P-450 are inducible by TCDD receptor ligands in mice (21). Due to the accumulation of radioactivity in the mucosa of the ethmoturbines after administration of radiolabeled TCDD and the presence of specific [3H]TCDD binding sites in this tissue it was considered of importance to investigate if a TCDD-inducible form of cytochrome P-450 was present in the mucosa. Since the presence of TCDD-binding sites suggests the presence of inducible cytochrome P-450 species, the ethmoturbinal fraction was further analyzed with respect to the presence of inducible cytochrome P-450 species.

The molecular weights of the inducible cytochrome P-450 species were estimated as A. 344,000 and B, 232,000.

Antibody Inhibition of Enzymatic Activities. Antibodies raised against purified cytochrome P-450 were utilized to investigate the nature of the 7-ethoxyresorufin O-deethylase activity of the mucosa of the ethmoturbines. In the presence of preimmune IgG the 7-ethoxyresorufin O-deethylase activity of the mucosa of the ethmoturbines was 92% of the control activity without antibodies. It was found that antibodies raised against BNF-induced cytochrome P-450 were equally or more active in inhibiting the activity of the ethmoturbinal and the pooled naso- and maxilloturbinal and nasal sepal particulate fractions did not increase appreciably [less than a 2-fold increase (Table 1)].

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Injections of BNF (80 mg/kg) or corn oil were administered i.p. to two groups of six rats each, 40 h prior to isolation of nasal tissues. Ethmoturbinates and livers were pooled from pairs of rats and naso- and maxilloturbinates together with the nasal septum were pooled from all six rats of each treatment group of an experiment. The experiment was repeated three times, n is the number of pools in each experiment. The nearly two-fold difference observed between the levels of enzymatic P-450d was present at detectable levels in the ethmoturbinates only of BNF-treated rats. The cytochrome P-450c mRNA but not for mRNA coding for cytochrome P-450d mRNA was present at detectable levels in the ethmoturbinates only after BNF administration (Fig. 7). In the liver, both mRNAs were strongly induced by BNF (Fig. 7).

**DISCUSSION**

Following administration of radiolabeled TCDD a selective uptake and retention of radioactivity in the mucosa of the ethmoturbinates of rats and mice was observed in addition to the previously known accumulation of TCDD in the liver (3, 6, 7). Earlier results suggest that TCDD is metabolized slowly in the rat and mouse (6, 23) and largely remains in its unchanged form in the tissues of exposed animals (6, 24).

TCDD is slowly metabolized in the rat liver to more polar products which are subsequently secreted into the bile, whereas unchanged TCDD does not appear in the bile (24). In agreement with previous work (24), a labeling of the bile and intestinal contents was observed also in the present investigation.

The high initial uptake of radioactivity in the adrenal cortex 1 h after injection of [14C]TCDD observed in this study is compatible with earlier results, since a transient localization of radioactivity in this tissue after administration of other radiolabeled lipophilic chlorinated compounds is a common finding (25, 26). The implications of this finding (for possible actions of TCDD in the adrenals) are not evident since the TCDD receptor has not been detected in this tissue (5), and adrenal aryl hydrocarbon hydroxylase activity is not inducible by TCDD receptor ligands, *i.e.*, 3-methylcholanthrene (27). However, TCDD is known to produce bleeding in rat adrenals (28).

In view of the thymotoxicity of TCDD (2) and the high cytosolic TCDD receptor concentration in the thymus (5) it is notable that no significant uptake in the thymus was observed in this and earlier investigations (6, 7). In concordance with these results, Lund *et al.* (29) have noted that the nuclear uptake of radioactivity observed in the thymus after injections of [3H]TCDD is only 6% of the corresponding uptake in the liver. However, it is possible that the thymic cells that are sensitive to TCDD constitute a restricted part of the cell population in the thymus. Recent studies indicate that epithelial cells with relatively high TCDD receptor levels (as compared to the total thymic cell population) constitute the primary target for TCDD in the thymus (30). In analogy to TCDD, corticosteroid hormones do not accumulate in the thymus, as judged by whole-body autoradiography (31), despite their well-known thymolytic effect. This effect may possibly reflect that other factors in addition to the intracellular concentrations of high-affinity receptors may govern tissue distribution (especially at higher doses) and that relatively large amounts of hormone have been used, as compared to physiological serum levels.

---

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pretreatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naso- and maxilloturbinates and nasal septum</td>
<td>Corn oil</td>
<td>42.1</td>
<td>27.5</td>
<td>86.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>BNF</td>
<td>77.9</td>
<td>48.7</td>
<td>98.5</td>
<td>1</td>
</tr>
<tr>
<td>Ethmoturbinates</td>
<td>Corn oil</td>
<td>167.1 ± 34.1*</td>
<td>139.4 ± 26.5</td>
<td>141.0 ± 11.2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>BNF</td>
<td>314.3 ± 53.3</td>
<td>229.5 ± 34.9</td>
<td>194.5 ± 16.0</td>
<td>3</td>
</tr>
<tr>
<td>Liver</td>
<td>Corn oil</td>
<td>177.7 ± 21.5</td>
<td>143.3 ± 17.2</td>
<td>87.9 ± 7.5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>BNF</td>
<td>3499.4 ± 616.5</td>
<td>2304.9 ± 59.4</td>
<td>2434.3 ± 877.6</td>
<td>3</td>
</tr>
</tbody>
</table>

* Mean ± SD.

---

Fig. 7. RNA blot hybridization of cytochromes P-450c and P-450d mRNA in ethmoturbinates and liver from BNF-treated rats, and ethmoturbinates from control rats. Total RNA was electrophoresed in 1.1% agarose-formaldehyde gels, blotted to nitrocellulose filters, and hybridized with cDNA probes specific for cytochrome P-450c (lanes a–c) or cytochrome P-450d (lanes d–f) mRNA. The positions of 28 and 18 S ribosomal RNA were determined by staining parallel lanes with acridine orange.
Since no tissue-bound radioactivity was detectable in autoradiograms of organic solvent extracted tissue-sections, the retention of radioactivity observed in the olfactory mucosa after administration of radiolabeled TCDD most probably is due to the presence of reversibly bound TCDD, or possibly metabolites thereof.

The mucosa of the nasal cavity is a complex tissue comprised of different structural components with several types of cells. The squamous, respiratory, and olfactory epithelia cover a connective tissue, the lamina propria. This connective tissue contains numerous subepithelial (Bowman’s) glands, blood vessels, and nerve bundles (32). It was not possible to determine the precise cellular localization of the radioactivity in the mucosa of the ethmoturbinates from the autoradiograms obtained in this study. The character of retention of TCDD in the olfactory mucosa resembles that of certain lipophilic steroid hormones which in analogy to TCDD become reversibly bound in the olfactory mucosa (33), in contrast to metabolites formed from a number of xenobiotics that become covalently bound in the nasal epithelium and Bowman’s glands (33, 34).

Another possible explanation of uptake of TCDD in the olfactory mucosa would be high levels of TCDD receptor in this tissue. The similar number of specific [3H]TCDD binding sites, $K_d$ of the binding, molecular weight of the binding species, and ligand-binding specificity of the TCDD binding species observed in the cytosol from ethmoturbinates and liver of the rat, together with the inducibility of BNF of cytochrome P-450d mRNA in both tissues, indicate that the specific [3H] TCDD binding in the cytosol from the ethmoturbinates is due to the presence of the TCDD receptor in this tissue.

Because the level of specific [3H]TCDD binding in the mucosa of the ethmoturbinates estimated in this study seemed comparable to that of other tissues where TCDD is not accumulated (5), it is not plausible that the presence of TCDD receptor in this tissue accounts for the in vivo accumulation of TCDD in the ethmoturbinates. Therefore the reason for the retention of TCDD in the olfactory mucosa remains unclear at present, as is the reason for the retention of TCDD in the liver. However, as compared to the liver tissue, the olfactory mucosa is a more complex tissue and contains several different types of cells (as outlined above). Thus, it cannot be excluded that high amounts of TCDD receptor are present in restricted cell populations in the nasal mucosa.

A third conceivable explanation for the retention of TCDD in olfactory tissues is binding to some high-capacity binding species for chlorinated compounds not detected in our assay. Such proteins are present in the rat prostate (35) and mouse and rat lung (36).

Due to the limited amounts of nasal tissue we have not attempted to investigate the subcellular distribution of radiolabeled TCDD in vivo in these tissues. Hence, it is possible that the radioactivity that is localized in the mucosa of the ethmoturbinates mainly represents TCDD that is reversibly bound in some cellular compartment other than the cytoplasm.

Several explanations are conceivable to account for the low inducibility of 7-ethoxyresorufin O-deethylase activity in mucosa of the ethmoturbinates in spite of the presence of specific [3H]TCDD binding sites in this tissue, e.g.: (a) BNF is not taken up in this tissue; (b) the gene for cytochrome P-450c is continuously repressed in this tissue; (c) increased amounts of cytochrome P-450c mRNA is produced but not translated here; and (d) the 7-ethoxyresorufin O-deethylase activity in this tissue is unrelated to cytochrome P-450c. In order to distinguish to some extent between these possibilities, we utilized cytochromes P-450c and P-450d cDNA probes to measure the levels of the corresponding mRNAs in the ethmoturbinates. That P-450d mRNA was detectable, as judged by RNA blot hybridization, in the ethmoturbinates only after BNF administration suggests (a) that the inducer indeed reaches the nasal tissues. The apparent absence of cytochrome P-450c mRNA in the ethmoturbinates even after BNF treatment may imply that the cytochrome P-450c gene is (b) silent or expressed at low levels, or (c) that the cytochrome P-450c mRNA stability is low in this tissue. To discern between these latter alternatives nuclear transcription experiments will have to be performed. The possible relationship (d) between the 7-ethoxyresorufin O-deethylase activity and cytochrome P-450 in the mucosa of the ethmoturbinates will be discussed in the following paragraphs.

To our knowledge the expression of cytochrome P-450d mRNA in the nasal tissue constitutes the first example of tissue-specific cytochrome P-450 gene expression where cytochrome P-450d mRNA but not cytochrome P-450c mRNA is present at measurable levels after pretreatment with BNF. In contrast, in the rat prostate, cytochrome P-450c mRNA but not cytochrome P-450d mRNA is induced to detectable levels by BNF administration. In the liver, both forms of mRNA are simultaneously induced by BNF treatment, although they appear to be independently regulated (15). Thus the nasal tissue may prove to be an interesting model system for the study of mechanisms of tissue-specific control of TCDD receptor-regulated gene expression.

There are several possible consequences and implications of the retention of radioactivity in the mucosa of the ethmoturbinates following administration of radiolabeled TCDD and the presence of the TCDD receptor in this tissue. In other tissues and cells, receptor binding of TCDD is associated with increased microsomal metabolism and/or toxicity as well as cellular proliferation and differentiation. The adult and fetal nasal mucosae have recently been established as sites capable of active metabolism of foreign and endogenous compounds (for recent reviews, see Refs. 33 and 37).

Our findings that the basal and BNF-inducible levels of activity of 7-ethoxyresorufin O-deethylase in the rat were higher in the mucosa of the ethmoturbinates [where the olfactory epithelium is located (32)] than in the pooled naso- and maxilloturbinal tissue [which contains respiratory epithelia (32)] and the nasal septum [which contains both respiratory and olfactory epithelia (32)] favor the notion that the olfactory mucosa is the major site of drug metabolism in the nasal tissues.

The low inducibility of 7-ethoxyresorufin O-deethylase activity by BNF observed in the present study is in accordance with earlier work, which was shown that no microsomal enzyme activity is inducible to any large extent in nasal tissues (37–39). It has been discussed whether the right inducer has not been found (37) or if the inducer does not reach these target tissues (38). However, our results indicate directly that TCDD is taken up in the nasal tissue and indirectly that BNF is taken up because the amount of cytochrome P-450d mRNA in the mucosa of the ethmoturbinates was increased by I.P. injection of BNF. Since the basal activity of 7-ethoxyresorufin O-deethylase of the mucosa of the ethmoturbinates was only marginally inhibited by antibodies raised against hepatic cytochrome P-450c, this activity may at least in part be dependent on enzymes other than cytochrome P-450c. Cytochrome P-450d purified

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P. Söderkvist et al., manuscript in preparation.
from rat liver is known to exhibit some 7-ethoxyresorufin O-deethylase activity (20).

The selective accumulation of TCDD in certain regions of the nasal mucosa may possibly be related to the etiology of nasal cancer (40). Long-term (2 years) feeding of TCDD to rats (28) increases the incidence of squamous cell carcinoma of the nasal turbinates and carcinoma of the liver. However, this effect was not observed in nasal tissues of mice or rats intubated with TCDD, or given acute i.v. administration of the compound. TCDD does not appear to be an initiator of liver carcinogenesis, since TCDD is not a potent mutagen (41), and the levels of covalent binding per mole of nucleotide extractable from liver plasma (53). Epidemiological studies have revealed that there was an increased incidence of tumors of the nasal region among woodworkers (54) and workers in the leather industry (55). There is also an increased risk of developing nasal and nasopharyngeal carcinoma of the ethmoturbinates.

A third possibility is that TCDD may act as a promoter in nasal tissues. Thus, TCDD enhances diethylnitrosamine carcinogenesis in rat liver (45), and the formation of 7,12-dimethylbenz(a)anthracene initiated skin papilloma in HRS/J hairless mice (46). A number of compounds are nasal carcinogens (33, 37, 47, 48) or are metabolized to nasal carcinogens in nasal tissues (49-51). Therefore, the possible role of TCDD and related compounds as cocarcinogens in nasal tissues may be relevant to investigate.

Impurities structurally related to TCDD, e.g., chlorinated diphenylethers, dibenzofurans, and dibenzodioxins have been found in commercial formulations of chlorophenols used as fungicides in sawmills (52). Chlorophenol formulations have also been used in the industry for impregnation of textiles and tanning of leather (53). Polychlorinated dibenzofurans and dibenzodioxins have been detected in blood from workers in the sawmill, textile, and leather industry at levels below 400 pg/g plasma (53). Epidemiological studies have revealed that there was an increased incidence of tumors of the nasal region among woodworkers (54) and workers in the leather industry (55). There is also an increased risk of developing nasal and nasopharyngeal cancer among woodworkers exposed to chlorophenols as compared to woodworkers not exposed to these chemicals, among whom the risk is nearly normal (56). Thus, impurities of chlorophenols resembling TCDD with regard to biological activity may have represented an exposure in common for some of the occupational groups at increased risk of nasal cancer, as pointed out by Hardell et al. (40).

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Uptake and Specific Binding of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Olfactory Mucosa of Mice and Rats

Mikael Gillner, Eva B. Brittebo, Ingvar Brandt, et al.


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