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# ONTOGENY OF THE CYTOSOLIC RECEPTOR FOR 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN RAT LIVER, LUNG, AND THYMUS

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The ontogeny of the cytosolic receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin was studied in Sprague-Dawley rats by quantitation of the receptor in hepatic, lung, and thymic cytosol. Concentrations of hepatic and lung cytosolic receptors increased rapidly after birth and remained at the highest levels from days 2 to 21. After this time, receptor levels in these tissues slowly declined with age. In the thymus, cytosolic receptor concentrations remained high from days 2 to 42 following birth. In these tissues and at all times examined, the receptors demonstrated very high affinities for [$^3$H]2,3,7,8-tetrachlorodibenzo-p-dioxin. From days 15 to 42 following birth, no consistent sex related differences in receptor content or affinity were observed in any of these tissues.

The polycyclic aromatic hydrocarbons such as 3-methylcholanthrene, benzo[a]pyrene, and TCDD[1] have been shown to be potent inducers of certain cytochrome P-450 associated monooxygenases as well as other enzyme activities in liver and nonhepatic tissues (1-4). The mechanism of induction appears to involve the stereo-specific, high affinity binding of these compounds to a cytosolic receptor protein (the Ah receptor), translocation of the ligand-receptor complex to the nucleus, and modulation of gene expression (5-9). The ontogenic expression of cytochrome P-450 associated AHH activity as well as the receptor in hepatic tissue has been reported (10). However, in this study concentrations of receptor were determined using a single concentration of [$^3$H] TCDD (10 nM) and assuming the relative affinity of the receptor for TCDD to be similar throughout development. In addition, recent evidence has indicated the presence of high concentrations of receptor in lung and thymic tissues from a variety of mammalian species (11, 12).

[1] Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AHH, aryl hydrocarbon hydroxylase; TCDF, 2,3,7,8-tetrachlorodibenzofuran.

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No endogenous ligand for the receptor has been found, and its physiological role is unknown. It is possible the function of this molecule may be age related, or dependent upon tissue specific development and differentiation. The results presented in this study compare the ontogeny of the receptor in rat liver, lung and thymus. Relative affinities of these receptors for TCDD at each age examined were determined.

## MATERIALS AND METHODS

Chemicals: [1,6-$^3$H] TCDD (sp. radioactivity 50.5 Ci/mmol) was synthesized and purified as previously described (13). TCDF was a gift of Dr. J.A. Moore (NIEHS, Research Triangle Park, NC). The remainder of the chemicals were purchased from sources cited previously (14).
Animals: Pregnant female rats were purchased from Charles River (Wilmington, MA). Upon arrival, the animals were fed commercial chow (Ralston Purina, Richmond, IN) and water ad libitum. Pregnant females were anesthetized by the use of ether. Neonatal rats were killed by decapitation. As indicated, tissues from a number of individual animals were pooled for a single analysis. Starting at day 15 following birth, males and females were analyzed separately.
Preparation of cytosol: Tissues were homogenized in 3 volumes of HEDG buffer (25 mM HEPES, 1.5 mM EDTA, 1 mM dithiothreitol, 10 percent glycerol (v/v)). The remainder of the cytosol preparation was carried out as previously described (14), and unless specified these and remaining procedures were carried out at 0°C. The cytosol was used within 1 h after preparation. The protein concentration of the cytosol was estimated by the absorbance at 215/225 nm (15), which was confirmed by the method of Lowry et al. (16).
Assay of specific binding of [$^3$H] TCDD using hydroxylapatite: Hydroxylapatite is used in this assay system to adsorb the [$^3$H] TCDD-receptor complex, while subsequent washes remove free and nonspecifically bound [$^3$H] TCDD. The incubation conditions used were similar to those previously described (14). One or two ml of the 105,000 x g supernatant fraction (2 to 4 mg protein/ml) were incubated with various concentrations (0.1 to 8.0 nM) of [$^3$H] TCDD and with [$^3$H] TCDD plus a 200-fold excess of TCDF. TCDF is a ligand which has very high affinity for the Ah receptor (14), but is more water soluble than TCDD. Thus, TCDF is used in this system to displace specifically bound [$^3$H] TCDD. These were incubated for 18 to 24 h at 0°C. The [$^3$H] TCDD and TCDF were added to the incubations in a volume of 5 µl 1,4-dioxane per ml of cytosol. The determinations of the specific binding of [$^3$H] TCDD using the hydroxylapatite assay system were carried out as previously described (14).
Data analysis: Radioactivity was determined using a Packard Tri-Carb Model 2450 scintillation spectrometer. Quenching was corrected by automatic external standardization. The counting efficiency for tritium ranged from 25-35 percent. Specific binding was determined as the difference between total binding (samples containing [$^3$H] TCDD) and nonspecific binding (samples containing [$^3$H] TCDD plus TCDF). When various concentrations of [$^3$H] TCDD were used, the number of binding sites (n; fmol per mg of cytosolic protein) and apparent equilibrium dissociation constants ($K_D$; nM) were determined by the method of Scatchard (17). Values for n and $K_D$ at days -5, -3, 5, 12, 28, 34, and 40 represent the mean of two separate experiments utilizing a number (3-34) of animals per age group per experiment. Values at days 2, 18, 15, and 20 are the mean ± S.D. of three separate experiments. Data for the tissue weights represent the mean value per animal of tissues collectively weighed. The student's t-test was used to evaluate differences between n or $K_D$ values at various time points.

## RESULTS

Figure 1 shows the age related concentrations of receptors as well as the apparent equilibrium dissociation constants for [$^3$H] TCDD binding in hepatic cytosol. In addition, the age related alterations in liver wet weights for these animals are shown. Hepatic cytosolic receptor levels were comparatively low in rat fetuses at days 5 and 3 prior to birth. At day 2 following birth, these levels were increased, and remained at a high level through day 21. Following this time, hepatic receptor concentrations appeared to gradually decline with age. High affinity binding of [$^3$H] TCDD to the receptor in liver was observed at all ages. $K_D$ values ranged from 0.12 to 0.65 nM. There were no statistical differences among $K_D$ values at days 2, 8, 15, and 21. The decline in hepatic receptor concentrations from day 28 onward was accompanied by an increase in the rate of hepatic tissue growth.

As in the liver, lower levels of the Ah receptor were observed in the lung prior to birth (Fig. 2). These levels increased following birth, reached a peak at day 8, and slowly declined with age. The highest concentrations of receptor in lung cytosol were observed between days 2 and 15. In this tissue,

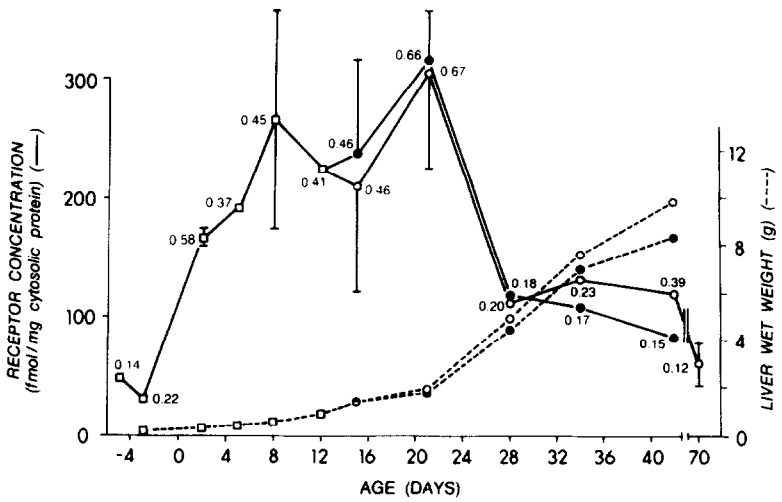


Figure 1. Hepatic cytosolic Ah receptor levels as a function of age in Sprague-Dawley rats (—). Each point represents the mean of two or three (where standard deviations are shown) separate experiments. Each determination was performed in duplicate using cytosol prepared from pooled tissues from 3 to 4 animals. From day 15 onward, male (o) and female (o) animals were separately determined.
For each age group and sex the $K_D$ values (nM) are given. The mean wet weight values (g) for the livers of each age group are also shown (---).

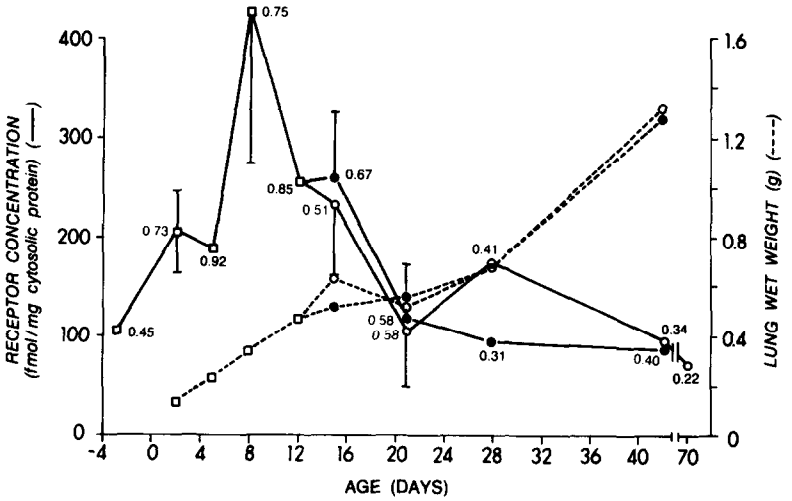


Figure 2. Lung cytosolic Ah receptor levels as a function of age. Conditions and symbols as in Figure 1.

$K_D$ values ranged from 0.22 to 0.95 nM. Again like the liver, there were no statistical differences among $K_D$ values at any time when three separate experiments were performed. During the period examined, the growth of the lung appeared to follow a nearly biphasic curve with relatively large growth rates occurring just after birth until day 15, and then again from days 28 to 42.

A relatively constant level of receptor for TCDD was observed in the thymus from all animals between 2 and 70 days of age (Fig. 3). Sufficient thymic tissue for analysis was not available from animals prior to birth.

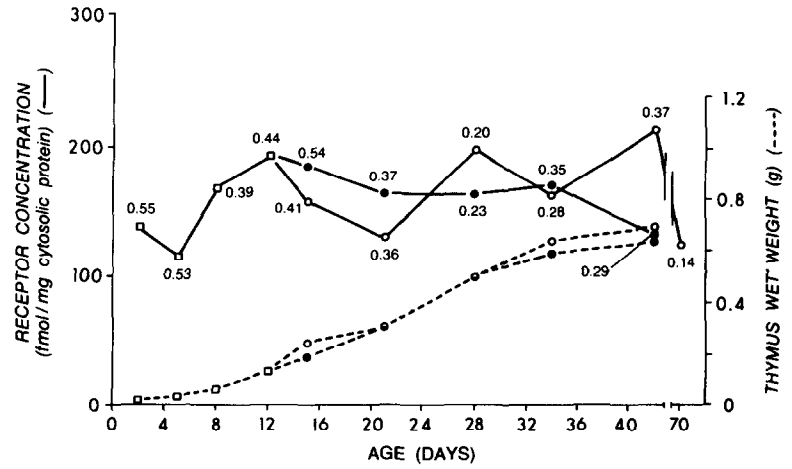


Figure 3. Thymic cytosolic Ah receptor levels as a function of age. Conditions and symbols as in Figure 1.

From days 2 through 70 following birth, the $K_D$ values (0.14 to 0.55 nM) obtained using thymic cytosol appeared to be more constant than observed in liver or lung. During the period examined, the maximum growth rate of the thymus occurred from days 8 to 34 following birth.

No consistent significant sex differences in receptor concentrations, $K_D$ values, or tissue weights were observed in liver, lung, or thymus during the period examined.

## DISCUSSION

The data obtained in this study concerning the ontogeny of the Ah receptor in rat hepatic cytosol are similar to that observed previously by Kahl et al. (10). Hepatic receptor concentrations were low or undetectable (10) prior to birth. These concentrations increased postnatally, reached peak levels between 8 and 21 days of age and then slowly declined throughout adulthood. A similar pattern has also been observed in hepatic tissue from rabbits and C57BL/6J mice (10). In the present study, an analogous age related alteration in the levels of cytosolic receptor was also observed in the lung. Receptor concentration in this tissue was the highest between days 2 and 15 following birth.

The age related pattern of hepatic Ah receptor concentrations, in general, paralleled maximal increases in basal and inducible levels of cytochrome P-450 and associated monooxygenase activities, including AHH (10). Notably, a similar pattern of AHH inducibility in rabbit lung has been observed (18). It is also of interest that the Clara cells, which contain the highest concentration of cytochrome P-450 in the lung (19), proliferate and differentiate mainly within the first few weeks of postnatal development of the rat and rabbit (20). In all species and tissues examined, the highest concentrations of receptors are localized in the liver, lung, intestine, and kidney (11,12). In general, these tissues also exhibit the highest levels of induced AHH activity (21). There are, however, some important qualifications to these parallels. Firstly, individual monooxygenase activities may exhibit very different developmental patterns (10) as compared to the Ah receptor.

Thus, the presence of the Ah receptor may be a necessary but not sufficient condition for the expression of these activities. It is possible that there are temporal controls which may regulate the expression of the structural genes. Secondly, in the guinea pig, low or no inducibility of AHH activity or cytochrome P-450 has been observed following TCDD (22) or 3-methylcholanthrene (23,24) exposure, despite the presence of a receptor molecule with similar properties as that observed in the rat (12,25). In addition, there are a number of other biochemical, biological, and toxicological responses to these compounds in a variety of tissues and species which are believed to be mediated by the Ah receptor (26). Thus, although many studies have focused mainly on the role of the receptor in the regulation of cytochrome P-450 associated monooxygenases, it seems clear that these are but a few of the many species and tissue specific responses controlled by the Ah locus (26).

Unlike the liver and lung, no consistent alterations in the concentrations or $K_D$ values for the Ah receptor were observed in the thymus from days 2 through 42 following birth. In the adult rat, the thymic cytosol contains the highest concentrations of receptor as compared to other tissues examined (11,12). Although TCDD-induced AHH activity in the thymus is less than 1/1000 that in the liver, in terms of percentage increase the thymic AHH activity is approximately twice as inducible as in the liver (27). In addition, thymic involution and impairment of thymus-dependent immune function are consistent features of TCDD exposure in most mammalian species (28). However, the exact relationship between the presence of the Ah receptor in the thymus and these TCDD-induced alterations is unknown.

The Ah receptors in the liver, lung, and thymus at all times examined in this study demonstrated very high affinities for [$^3$H] TCDD. This suggests, but does not prove, the existence of a single class of binding sites in these tissues throughout the course of development. If this is the case, other factors such as activation and/or disposition of the receptor may influence the differential temporal expression of various gene products. Alternatively, it is possible that noted, although not significant, variations in the

relative $K_D$ values at different times may reflect the presence of a subpopulation(s) of receptor sites with varying degrees of specificity towards different ligands. This heterogeneity of binding sites for TCDD has been postulated, and would support data showing varied developmental patterns of specific monooxygenase activities (10). Further purification of these receptor molecules would assist in differentiating these possibilities.

At present the functional role of the receptor molecule remains to be elucidated. The similarity of the ontogeny of the receptor in hepatic cytosol from a number of mammalian species (10), suggests a conservation of a functional role of the receptor in this tissue. Although it is of interest that in the liver and lung, changes in the concentration of these receptors generally correspond to birth as well as the approximate time of weaning, this pattern was not apparent in the thymus. In an additional study, Dencker and Pratt (29) observed that in the day 13 embryo of the mouse, the concentration of receptors was higher in the maxillary process of the palate than other tissues including liver, brain, limb buds, and skin. This relationship appears to correspond with the time of development of the palate as well as the ability of TCDD to cause cleft palate in this species. Thus, the concentration of receptor in any particular tissue may depend on the function, if any, of the receptor in the development of that tissue.

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