Ah Receptor in Human Placenta: Stabilization by Molybdate and Characterization of Binding of 2,3,7,8-Tetrachlorodibenzop-dioxin, 3-Methylcholanthrene, and Benzo(a)pyrene

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The human placenta is remarkably responsive to induction of AHH when exposed to certain xenobiotic chemicals. Placental microsomes from women who smoke have AHH activities that typically are 10 to 20 times higher than microsomes from nonsmokers (1-6). Placental AHH is highly inducible in several human cells and tissues exposed to specific halogenated and nonhalogenated aromatic chemicals of the "3-methylcholanthrene-type." In laboratory animals AHH induction is known to be regulated by binding of inducers to the Ah receptor, a soluble intracellular protein. However, the induction mechanism in the human species is incompletely understood largely because the Ah receptor, which seems essential to the induction process, has not previously been detectable in certain human cells and tissues (including placenta) that are responsive to AHH induction. We now report that human placenta contains high concentrations of Ah receptor (comparable to the receptor concentrations in rat and mouse liver) but that special modifications were necessary in the assay techniques in order to detect and accurately quantitate receptor binding. Receptor was detected at concentrations ≥100 fmol/mg cytosol protein using [3H]2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as the radioligand. This high concentration of specific binding was present only if the placental tissue was initially homogenized in a buffer containing sodium molybdate (10 or 20 mM). Without molybdate in the homogenizing buffer, specific [3H]TCDD binding was about only 35 fmol/mg. Specific Ah receptor binding also was detectable with [3H]-3-methylcholanthrene and, to a lesser extent, with [3H]-benzo(a)pyrene. The receptor sedimented near 9S on sucrose gradients whether molybdate was present or not. About 80% of specific binding was lost if excessive charcoal was used to adsorb "nonspecifically bound" ligand from cytosol prior to gradient analyses. The apparent affinity with which [3H]TCDD bound to Ah receptor in human placental cytosol was relatively low (apparent Kd = 5 to 8 nm) when compared with the affinity of [3H]TCDD binding in rat or mouse hepatic cytosols (Kd = 1 to 3 nm). These data suggest that while molybdate has very little effect on the quantity or molecular size of the rodent Ah receptor assay, it is very important in stabilizing the human Ah receptor. Our experiments demonstrate that human placenta contains a high concentration of Ah receptor and suggest that AHH induction in placenta is mediated through a receptor mechanism analogous to that previously established in tissues and cells from laboratory animals.

INTRODUCTION

The human placenta is remarkably responsive to induction of AHH when exposed to certain xenobiotic chemicals. Placental microsomes from women who smoke have AHH activities that typically are 10 to 20 times higher than microsomes from nonsmokers (1-6). Placental AHH also is highly inducible when women have been exposed to mixtures of polychlorinated biphenyls, quaterphenyls, and dibenzoferans (6); the induced AHH activity correlates with increased levels of a cytochrome P-450 isozyme corresponding to cytochrome P-4505 (7).

AHH is a key enzyme activity both in the "metabolic activation" and in the "detoxication" of potentially toxic, mutagenic, and carcinogenic environmental compounds, especially polycyclic aromatic hydrocarbons such as benzo(a)pyrene (8-11). Thus the mechanism by which AHH induction is regulated is of considerable interest in understanding the responses of human tissues to toxic and carcinogenic agents.

In tissues from laboratory animals AHH induction by polycyclic aromatic hydrocarbons and polychlorinated aromatic compounds is initiated by their specific binding to a soluble intracellular protein, the Ah (aromatic hydrocarbon) receptor (12-16). Ligands for the Ah receptor (and, hence, inducers of AHH) include TCDD, MC, BP (12, 14, 17), and certain dibenzoferans and coplanar polychlorinated biphenyls (18). The ultimate site of action of the Ah receptor-ligand complex is within the nucleus where binding of the complex to specific gene regions leads to enhanced transcription of cytochrome P-450 mRNA (12, 13).

Although several human cells in culture as well as some human tissues in vivo have been shown to exhibit AHH induction (10, 19), there has been little direct evidence that responsive human cells or tissues contain a receptor equivalent to the Ah receptor which has been extensively characterized in rodents and other laboratory species (20, 21). Until recently it had appeared that the Ah receptor was either absent from human placenta or present at very low, near nondetectable, levels (22-24).

We now report that human placenta has high levels of Ah receptor. However, the human placental Ah receptor has lower affinity for its ligands and is less stable than the receptor in rodent tissues. We describe technical modifications which are...
MATERIALS AND METHODS

Chemicals and Reagents. Some experiments were done with [3H]-TCDD (50 Ci/mmol) purchased from ICN Chemical and Radioisotopes Division (Irvine, CA); this [3H]TCDD was repurified in our laboratory to greater than 95% radiochemical purity by HPLC as previously described (25). Another batch of [3H]TCDD (32 Ci/mmoll and nonradioactive TCDD was generous gifts from Dr. S. Safe (Texas A & M University) who synthesized the [3H]TCDD and TCDBF. This [3H]TCDD was of greater than 95% radiochemical purity when analyzed in our laboratory by HPLC. [3H]MC (generally labeled, 37 Ci/mmoll from Amersham Corp. (Oakville, Ontario, Canada) and [3H]BP (55 Ci/mmoll) from New England Nuclear-Dupont Canada (Durval, Quebec, Canada) were repurified by reverse phase HPLC to greater than 97% radiochemical purity in our laboratory. Nonradioactive MC was purchased from Eastman Organic Chemicals (Rochester, NY); nonradioactive DB[a,h]A, BP, dextran (average M, = 86,900), dithiothreitol, dexamethasone, 17β-estradiol, and progesterone from Sigma Chemical Co. (St. Louis, MO); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid from Calbiochem-Behring Corp. (La Jolla, CA); sucrose (density gradient grade) from Beckman Instruments (Toronto, Ontario, Canada); dimethyl sulfoxide, charcoal (Norit A), sodium molybdate, and EDTA from Fisher Chemical Co. (Toronto, Ontario, Canada).

Placental Tissue. Full-term placentas were collected at delivery and immediately placed in ice-cold saline. All subsequent procedures were done at 0–4°C. Fetal villous tissue was sampled from the maternal decidua basalis, rinsed free of blood, and homogenized for 30 s in 5 ml 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-1.5 mM EDTA-1 mM dithiothreitol-10% glycerol (v/v), pH 7.6 buffer or the same buffer plus sodium molybdate per g tissue in a cooled Waring blendor.

Preparation of Cytosol. The placental homogenate was centrifuged at 4,000 x g for 10 min, and the supernatant from this was centrifuged at 10,000 × g for 20 min. The 10,000 × g supernatant was centrifuged at 105,000 × g for 1 h to yield a supernatant cytosol fraction. Cytosol aliquots were immediately frozen in liquid nitrogen and then shipped on dry ice from Denver to Toronto. Specimens then were refrozen in liquid nitrogen until the time of receptor analysis. Some placental specimens, obtained immediately at delivery by caesarian section in Toronto, were used to test the effect of freezing and storage on the stability of the Ah receptor. Protein concentrations in the cytosols were determined by the method of Bradford (26) using Bio-Rad reagents (Bio-Rad Laboratories, Mississauga, Ontario, Canada) with bovine serum albumin as the standard.

Incubation of Cytosol with Ligands. The basic procedure will be outlined here. Procedures were modified in particular experiments as indicated in the legends to figures and tables. Placental cytosols (0.5 ml; approximately 5 mg protein/ml) were incubated with a radioligand ([3H]TCDD, [3H]MC, or [3H]BP) for 1 h at 0–4°C. The radioligands were added to cytosol in 10 μl of dimethyl sulfoxide/ml cytosol; dimethyl sulfoxide also was used as the solvent to add nonradioactive chemicals used as competitors. To test specificity of the radioligand binding, each sample of cytosol was incubated with radioligand plus solvent only, and in a parallel incubation with an excess (ordinarily a 100-fold m excess) of a nonradioactive competitor. Competitors used included nonradioactive TCDD, TCBF, MC, and DB(a,h)A, all of which previously have been shown to be potent Ah-receptor agonists (14, 16–18). After incubation, unbound and loosely bound ligands were removed by adding cytosol samples to a charcoal-dextran pellet. The charcoal-dextran was resuspended on a Vortex mixer, then removed by centrifugation at 4000 × g for 15 min.

Velocity Sedimentation on Sucrose Gradients. Labeled cytosol samples were analyzed by density gradient centrifugation using the vertical-tube rotor technique described by Tsui and Okey (27). Aliquots (300 μl) of charcoal-treated cytosol were layered onto linear (10 to 30%) sucrose gradients prepared in the same buffer in which that particular cytosolic sample had been prepared. Gradients were centrifuged at 2°C for 2 h at 372,000 × g av. After centrifugation, 25 fractions (200 μl each) were collected from each gradient using an ISCO Model 640 gradient fractionator (Instrumentation Specialties Co., Lincoln, NE). Sedimentation coefficients (s20,w) for radioactive peaks were calculated by the method of Martin and Ames (28) relative to [3H]formaldehyde-labeled bovine serum albumin (4.4S) and [3H]formaldehyde-labeled catalase (11.3S) which were included in each gradient as internal sedimentation markers. Radioactivity in each fraction was determined by liquid scintillation counting and was corrected for counting efficiency.

RESULTS

Effect of Molybdate on [3H]TCDD Binding in Placental Cytosol

Placental cytosol, prepared in buffer without molybdate, exhibited a small [3H]TCDD binding peak that sedimented near 9S in sucrose gradients (Fig. 1A). The 9S peak was much larger when tissue samples from the same placenta were homogenized in buffer containing 20 mM sodium molybdate (Fig. 1B). Fig. 1C illustrates a placental cytosolic specimen in which a particularly large peak of [3H]TCDD binding in the 9S region was detected with molybdate present in the homogenizing buffer.

For comparison with [3H]TCDD binding, we also tested the effect of molybdate on glucocorticoid receptor in human placental cytosol using [3H]triamcinolone acetonide as the radioligand. Without molybdate in the homogenizing buffer, no specific binding of [3H]triamcinolone acetonide to glucocorticoid receptor could be detected in placental cytosol; when portions of the same placenta were homogenized in buffer containing 20 mM molybdate, a small specific binding peak (10 fmol/mg cytosol protein) for [3H]triamcinolone acetonide was present and sedimented ≈8S (data not shown).

Specificity of Binding

If the 9S binding component in human placental cytosol represents the Ah receptor, it should bind specifically those chemicals that are known to be Ah-receptor agonists. As shown in Fig. 1, incubation of cytosol with [3H]TCDD in the presence of nonradioactive TCBF (a potent Ah-receptor agonist in rodents) completely inhibited [3H]TCDD binding in the 9S region; binding of [3H]TCDD in other regions of the gradient was unaffected by TCBF.

Fig. 2 further illustrates that the 9S peak is specific for those compounds known to be Ah-receptor agonists in rodents. Nonradioactive TCDD and MC, both known agonists for rodent Ah receptor, eliminated binding of [3H]TCDD in the 9S region; another Ah receptor agonist, DB(a,h)A, also completely inhibited [3H]TCDD binding in the 9S region (data not shown). In contrast, the steroid hormones 17β-estradiol and progesterone did not affect binding of [3H]TCDD in the 9S region nor did the synthetic glucocorticoid, dexamethasone (Fig. 2). Binding of [3H]TCDD in the 9S region also was not affected by phenobarbital (data not shown).

Specific binding of Ah-receptor agonists in the 9S region also could be demonstrated directly with [3H]MC and [3H]BP as the radioligands (Fig. 3). The concentration of specific binding sites detected with [3H]MC as the radioligand (Fig. 3) was approximately the same as that detected with [3H]TCDD in the same cytosol (shown in Fig. 2). Some specific binding of [3H]BP was detectable in the 9S region (Fig. 3); however, the concentration of specific binding sites detected with [3H]BP was only about 10% of that detected with either [3H]TCDD or [3H]MC.

The 9S binding peak was the only distinct peak that was necessary in the assay in order to detect and characterize Ah receptor in human placenta.
third of the gradients, no distinct specific or nonspecific peaks were observed in the 4 to 5S region nor in other regions of the gradient (Fig. 3).

The selective binding of a spectrum of known Ah-receptor agonists to the 9S binding component in human placental cytosol establishes that the 9S component meets the specificity criteria expected of the Ah receptor.

Fig. 2. Specificity of [3H]TCDD binding in human placental cytosol. Cytosol [5 mg protein/ml, prepared in 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-1.5 mM EDTA-1 mM diithiothreitol-10% glycerol (v/v), pH 7.6-sodium molybdate buffer] was incubated with 10 nM [3H]TCDD in the absence of competitors (C), in the presence of compounds that are known agonists for the Ah receptor in rodent tissues (top), or in the presence of various steroid hormone analogues (bottom). Samples were analyzed on sucrose gradients as described in "Materials and Methods." DEX, dexamethasone; E2, 17β-estradiol; PROG, progesterone; BSA, bovine serum albumin; Cat, [14C]formaldehyde-labeled catalase. Arrows as in Fig. 1.

Sedimentation Properties of the Specific Binding Component

When placental cytosol was prepared in 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-1.5 mM EDTA-1 mM diithiothreitol-10% glycerol (v/v), pH 7.6-sodium molybdate buffer containing 10 or 20 mM molybdate, the specific binding peaks sedimented at ≈9S (Figs. 1–3; Table 1). Although the concentration of specific binding sites was reduced in the absence of molybdate, the sedimentation position of the residual receptor population stayed at ≈9S (Fig. 1; Table 1). Under conditions of high ionic strength in the absence of molybdate (i.e., in 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-1.5 mM EDTA-1 mM diithiothreitol-10% glycerol (v/v), pH 7.6 buffer containing 0.4 M KCl), specific binding of [3H]TCDD to the Ah receptor was completely abolished (data not shown). If cytosol was prepared in buffer which contained molybdate, 0.4 M KCl did not decrease specific binding of [3H]TCDD and the [3H]TCDD-receptor complex continued to sediment in the 9S position (data not shown).

Optimal Storage and Assay Conditions

Preparation and Storage of Placental Cytosol. It was important to determine the conditions under which placental cytosols could be prepared, shipped, and stored without loss of specific binding activity. If placental tissue was homogenized in buffer which contained molybdate, subsequent freezing in liquid nitrogen and re thawing for assay did not reduce the concentration of specific binding sites. This was assessed by preparing cytosol and analyzing some aliquots on the same day without any freezing; specific [3H]TCDD binding in aliquots that were frozen in liquid nitrogen before assay was the same as that in
cytosol that never had been frozen (data not shown). Specific binding was stable in cytosols stored in liquid nitrogen for periods up to 3 months; we did not test stability in liquid nitrogen beyond 3 months.

In cytosols that contained molybdate, specific binding also was stable for at least 3 months at −80°C and was stable for 2 weeks at −20°C; after 3 months at −20°C, all specific binding was lost, even with molybdate present in the cytosol. Binding also was stable in cytosols that were initially frozen in liquid nitrogen, shipped on dry ice for 3 days, and then refrozen in liquid nitrogen (data not shown).

Molybdate was effective at stabilizing placental Ah receptor only if molybdate was present in the initial homogenizing buffer. If molybdate addition was delayed until after the cytosol had already been prepared, the concentration of Ah receptor detected was the same as the low concentration detected in samples prepared and analyzed totally in the absence of molybdate (data not shown).

The concentration of Ah receptor detectable in placental samples prepared in buffer which contained 20 mM molybdate was slightly higher than in samples prepared with 10 mM molybdate, but the difference between receptor concentrations detected in buffer containing 10 versus 20 mM molybdate was not statistically significant (Table 2). The concentration of Ah receptor detected in placental samples homogenized in buffers containing 30 to 100 mM molybdate was no greater than that of samples homogenized in buffer containing 20 mM molybdate (data not shown).

Conditions for Incubation with Radioligand. Experiments were conducted on cytosol samples from several placentas to determine what concentration of [3H]TCDD was required to saturate specific binding sites and to determine what length of incubation of cytosol with [3H]TCDD would detect the maximum concentration of specific binding sites. At all concentrations of molybdate tested (0, 10, and 20 mM) the concentration of receptor sites detected was higher when cytosol was incubated with 20 nM [3H]TCDD than when the same cytosols were incubated with 10 nM [3H]TCDD. By analysis of variance, when all samples analyzed at 10 nM [3H]TCDD were compared with all samples analyzed at 20 nM [3H]TCDD, the difference was statistically significant (P < 0.01); however, at any single concentration of molybdate the difference between 10 and 20 nM [3H]TCDD was not significant (P > 0.05) (Table 2). In most cases, the concentration of receptor sites detected also was slightly higher if cytosol was incubated with [3H]TCDD for 2 h at 4°C rather than for 1 h; however, the difference in the mean concentration of sites detected for the group of eight cytosolic preparations was not significantly higher at 2 than at 1 h (data not shown). We tested the effect of prolonged incubation with [3H]TCDD on the concentration of receptor detected in a small number of different cytosolic specimens. Binding was not increased by incubating cytosols with [3H]TCDD for periods longer than 2 h at 4°C. On the other hand, specific binding capacity in placental cytosol also was not lost during prolonged incubation with [3H]TCDD at 4°C. If cytosol contained both molybdate and [3H]TCDD, little specific binding was lost over periods as long as 24 h at 4°C. In the absence of [3H]TCDD, about one-half the specific binding capacity of the cytosol was lost after 24 h at 4°C, even with molybdate present in the buffer (data not shown).

Effect of Charcoal. Detection of maximal levels of Ah receptor in placental cytosol was critically dependent upon the amount of charcoal-dextran used to adsorb "nonspecifically bound" ligand prior to the separations on sucrose gradients. If charcoal-dextran was added to placental cytosol at amounts greater than 0.1 mg charcoal/mg cytosol protein, specific binding of [3H]-TCDD to the Ah receptor was greatly reduced; the loss was even more pronounced as the amount of charcoal added was increased (Fig. 4). At the standard amount of charcoal-dextran that we previously have used in most experiments with cytosols from laboratory animals (≈2 mg charcoal/mg cytosol protein),
only about 20% of specific binding of [3H]TCDD to Ah receptor remained in human placental cytosols; in contrast, stripping of specifically bound [3H]TCDD from Ah receptor in rat hepatic cytosol occurred only when charcoal concentrations were increased to more than 10 times the level that caused loss of specific binding in placental cytosols (Fig. 4). If charcoal treatment was completely omitted from the procedures, the background radioactivity in profiles was high, making interpretation difficult. A level of ~0.1 mg charcoal-dextran/mg cytosol protein provides a balance between an unacceptably high background radioactivity and excessive "stripping" of specific binding from the receptor.

Precision of the Sucrose Gradient Assay for Ah Receptor in Placental Cytosol. The coefficient of variation in the assay was approximately 10%, both for replicate assays done on the same cytosol on the same day and for multiple assays done on the same cytosol over a period of 3 days (data not shown).

Affinity of Binding

Saturation of the Ah receptor in the human placental cytosols that were tested required incubation with higher concentrations of [3H]TCDD than is required in cytosols from tissues or cells from laboratory animals (Fig. 5). The apparent $K_a$ for specific binding of [3H]TCDD to Ah receptor in placental cytosols from three different donors ranged from 5.5 to 7.9 nM (Table 3; Fig. 6) compared with a $K_a$ of 2.4 nM for rat hepatic cytosol analyzed by the same procedure (Table 3).

Since the sucrose gradient assay for detection and quantitation of Ah receptor is relatively laborious and expensive, we attempted to detect and quantitate specific binding of [3H]TCDD in placental cytosols by the more rapid and economical hydroxylapatite adsorption assay (29). Although the hydroxylapatite assay works well with rodent cytosols (29–31), the results with placental cytosols were very inconsistent. Specific binding of [3H]TCDD in placental cytosols seems to be rapidly lost during the detergent-washing procedures that are necessary to remove nonspecifically bound [3H]TCDD in the hydroxylapatite assay (data not shown).

**DISCUSSION**

There is considerable evidence to support the Ah receptor's role in regulation of cytochrome P450 induction in tissues of...
laboratory animals in vivo (12) and in mouse hepatoma cells in culture (13, 32). Hudson et al. (33) reported low levels of Ah receptor in human lung, culture (13, 32). Hudson et al. (33) reported low levels of Ah responsive tissues from laboratory animals. Our early experiments with placenta, however, all were done with the same techniques that we previously had used successfully on tissues from laboratory species. We conclude that those procedures, per se, are not suitable for identifying and accurately quantitating Ah receptor in human placenta.

However, using the modified procedures described in this paper, we have now established that human placenta contains high concentrations of Ah receptor. The specific 9S binding peaks illustrated in Figs. 1–3 appear small because these plots include data points at the top of the gradients where nonspecific background radioactivity is high. In fact, the concentration of Ah receptor detected in human placental cytosols (mean of 106 fmol/mg protein in placentas from 10 individuals, Table 2) is equal to or higher than the concentration of receptor commonly detected in hepatic cytosols from mice or rats (14, 15, 17, 29). Since it is very difficult to remove blood from placental samples, it is probable that our assays underestimate the true concentration of Ah receptor that is present in placental tissue.

Another major factor making it difficult to detect and quantitate the Ah receptor in human placental cytosol is the relatively low affinity with which placental receptor binds its ligands. In our previous studies, using the same analytical procedures, the apparent $K_d$ for binding of [3H]TCDD to Ah receptor ranged from 0.7 nM in hepatic cytosol from C57BL/6N mice (15) to 2.9 nM in hepatic cytosol from cynomolgus monkeys (35). In the present study the apparent affinity with which [3H]TCDD bound to placental Ah receptor ($K_d = 5 \text{ to } 8 \text{ nM}$) was weaker than that in rodent species. This lower affinity is manifested by loss of specifically bound [3H]TCDD when placental cytosols are treated with charcoal-dextran to remove nonspecifically bound ligand. Our observations support the previous hypothesis by Jaiswal et al. (20) that the reason why cytosolic Ah receptor was not detectable in human MCF-7 cells might be that these cells contain a “low-affinity form” of Ah receptor. In addition to detecting Ah receptor in human placenta, our laboratory also has discovered that the human A431 squamous cell carcinoma line has substantial levels of cytosolic Ah receptor; however, the A431 receptor, like the human placental receptor, has a markedly reduced affinity for [3H]TCDD when compared with rodent cytosolic receptors. Inbred strains of mice that are “genetically responsive” to cytochrome P-450 induction by 3-methylcholanthrene appear to have a “high-affinity” form of Ah receptor whereas “genetically nonresponsive” strains have a low-affinity receptor, possibly as the result of a mutation at the Ah locus which encodes the receptor protein (12). Differences in receptor affinity between mice lead to dramatic differences in their susceptibility to toxic and carcinogenic effects of several polycyclic aromatic hydrocarbons and halogenated aromatic compounds (10). It is not yet known whether the human population is similarly divided into individuals with high-affinity versus low-affinity Ah receptors. Currently we are undertaking studies of placental tissue from a large number of individuals to determine the degree of receptor heterogeneity within the human population. In the small population studied to date the apparent $K_d$ values cluster in the 5 to 10 nM range, and we have no evidence yet of significant differences in receptor amount or affinity among individuals in the human population.

Reduced affinity for [3H]TCDD, if it proves to be a general property of human Ah receptor, may partially explain why humans seem to be less sensitive to the toxic effects of TCDD (and related halogenated compounds) than are most laboratory species.

It should be mentioned here that although the Ah receptor

![Figure 6. Determination of apparent affinity of [3H]TCDD binding in human placental cytosol by Scatchard plot analysis. Cytosol aliquots (5 mg protein/ml from specimen 8.1C) were incubated with [3H]TCDD at concentrations ranging from 1 to 40 nM for 2 h at 0-4°C. Aliquots then were treated with charcoal-dextran (0.1 mg/mg cytosol protein), and specific binding was determined for each sample by analysis on sucrose gradients. “Unbound” [3H]TCDD is defined as [3H]TCDD not specifically bound to the 9S receptor peak. The Scatchard plot (bottom) was derived from the saturation plot shown at top. Binding parameters in the Scatchard plot were calculated by least-squares linear regression.](image)
previously has been undetectable in cytosol from genetically nonresponsive strains of mice, the techniques we describe for human placenta reveal that significant amounts of apparent low-affinity Ah receptor are present in hepatic cytosol from nonresponsive strains of mice. It is also noteworthy that we were able to detect Ah receptor in human placental cytosol by direct binding of both [3H]MC and [3H]BP and that the concentration of receptor detected by [3H]MC was equivalent to that detected with [3H]TCDD. Our previous studies in rat and mouse hepatic cytosols indicated that [3H]MC bound to Ah receptor with essentially the same affinity as [3H]TCDD (36). Other investigators, however, reported that by competition studies between nonradioactive MC and [3H]TCDD, MC binds to Ah receptor in rodent cytosols with an affinity about 1 log unit weaker than that of TCDD (14, 29). The clear binding of [3H]MC to Ah receptor that we observed in human placental cytosol may indicate that the placental Ah receptor binds nonhalogenated agonists more effectively (relative to TCDD) than do the receptors from rodent tissues. The great sensitivity of placenta to AHH induction in smokers might be a reflection of a high relative affinity and efficacy with which human placental receptor binds nonhalogenated polycyclic aromatic hydrocarbons. The relative affinity of halogenated and nonhalogenated agonists for Ah receptor in human placental cytosol is under further study in our laboratory.

Another major difference between human placental Ah receptor and the Ah receptor from rodents resides in the effect of molybdate on receptor stability. In our previous studies (31) on Ah receptor in hepatic cytosols from rats and mice, we found that molybdate slightly retarded the rate of thermal inactivation of the unoccupied receptor. However, addition of molybdate to the homogenizing buffer did not increase the level of detectable Ah receptor in mouse or rat hepatic cytosol over that seen without molybdate (31). In contrast, our present studies with human placental cytosol show that inclusion of molybdate in the homogenizing buffer has a dramatic effect on the concentration of Ah receptor subsequently detected. Molybdate also reduces the rate of inactivation of placental cytosolic Ah receptor after the receptor is occupied by ligand.

Molybdate previously has been shown by several laboratories to be highly effective at stabilizing steroid-hormone receptors, especially the glucocorticoid receptor (37, 38). The mechanism by which molybdate acts to stabilize cytosolic receptors is not yet clear. It has been proposed that molybdate acts to stabilize receptors by partially inhibiting receptor degradation by proteases (38). Wong et al. (24) added several protease inhibitors to the homogenizing buffer which they used to prepare placental cytosols, but no placental Ah receptor could be detected either with or without the protease inhibitors. They also found that mixing of human placental cytosol with rat hepatic cytosol did not destroy the rat cytosolic Ah receptor. Thus, human placental cytosol does not appear to contain potent proteolytic enzymes that might rapidly degrade Ah receptor under conditions of the standard assay. It seems, therefore, that the stabilizing action of molybdate on cytosolic receptor from human placenta is exerted by means other than inhibition of proteolytic enzymes.

The major alternative mode of action proposed for molybdate (other than inhibition of proteolysis) is inhibition of dephosphorylation of receptor protein, thereby maintaining a receptor conformation that prevents oxidation of sulfhydryl groups (37). We found previously that cytosolic Ah receptors from mouse and rat liver contain reactive sulfhydryl groups whose intensity is essential for the receptor to bind [3H]TCDD (39). Although the placental Ah receptor likely also contains reactive sulfhydryl groups, it is not yet possible to determine whether inhibition of dephosphorylation and stabilization of sulfhydryl groups is the main mechanism by which molybdate acts to preserve placental Ah receptor.

In sum, the main technical requirements for detecting, quantitating, and characterizing Ah receptor in human placental cytosol are (a) inclusion of molybdate in the homogenizing buffer and throughout subsequent analyses, (b) reduction of the amount of charcoal used so that specific binding of [3H]TCDD to Ah receptor will not be stripped along with the nonspecific binding, and (c) use of higher concentrations of [3H]TCDD than those used in rodents so that the relatively low-affinity human receptor will approach saturation during incubation with radioligand.

With these procedures the human placenta is seen to be a rich source of Ah receptor. Whether the Ah receptor in human placenta plays a role in some process other than regulation of AHH induction is not known. The assay procedures outlined in this paper may aid in the identification of Ah receptor in other human tissues and cells and may assist in the determination of the receptor’s overall biological function.

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