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Induction of UDP-Glucuronosyltransferase Activity in the Reuber H-4-II-E Hepatoma Cell Culture

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SUMMARY

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The rat Reuber H-4-II-E continuous cell line contains 4-methylumbelliferone-UDP-glucuronosyltransferase activity. This enzyme is induced 3- to 6-fold by polycyclic aromatic compounds such as benzo[a]pyrene, 3-methylcholanthrene, benz[a]anthracene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, by phenobarbital, or by isoproterenol. Induction of the transferase by most compounds is maximal by 48 hours; however, upon treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or 3-methylcholanthrene, the activity rises for at least 72 hours. Certain hydroxylated metabolities of benzo[a]pyrene, at the same concentration as used for benzo[a]pyrene, are shown to be inferior inducers of activity. Available hydroxylated metabolites of benzo[a]anthracene or 3-methylcholanthrene do not induce transferase activity. Combinations of a polycyclic aromatic compound and phenobarbital—at their optimal concentrations when added individually—cause additive induction of transferase activity whereas combinations of two polycyclic aromatic compounds do not cause greater activity than the best inducer alone. The induction of activity by the best inducer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, is progressively inhibited by increasing concentrations of either actinomycin D or cycloheximide. In contrast, if cells are first exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and cycloheximide for a period of time and then washed, treatment with actinomycin D causes an unusually rapid rise in transferase activity, as compared with that in cells transferred to control medium. The Reuber H-4-II-E cultures therefore contain a UDP-glucuronosyltransferase activity which is inducible by the three different classes of inducers already known to exist for monooxygenase activity in cell culture (2). Furthermore, substrates for UDP-glucuronosyltransferase(s) do not appear to function as very efficient inducers of the transferase activity.

INTRODUCTION

UDP-glucuronosyltransferase(s) (EC 2.4.1.17) is a membrane-bound enzyme which functionally catalyzes synthetic reactions involving conjugation during drug detoxification. Although the number of substrate specificities of the transferases is not delineated, this general class of enzymes can conjugate a variety of endogenous lipophilic compounds already containing an appropriate substituent group as in hydroxy or carboxy derivatives. On the other hand, certain lipophilic compounds may acquire an oxygen-containing group by membrane-bound cytochrome P-450-dependent

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monooxygenase. Appropriately substituted compounds are conjugated to glucuronic acid and rendered more water soluble and excretable. As enzyme systems in the drug-metabolizing pathway of the endoplasmic reticulum, both the monooxygenase(s) and transferase(s) activities increase *in vivo* after treatment with an inducer-compound from two different classes (3–5). The concomitant or sequential rise in activities after treatment with a single compound suggests that a common signal or mediator serves to maintain or enhance concerted actions of the two enzymes.

The basis for the coordinate increase in a monooxygenase and a UDP-glucuronosyltransferase enzyme in drug metabolism is not understood. Whether the two enzymes rise concomitantly or sequentially is also not well documented. It is known that both enzyme activities are induced by the *in vivo* administration of either phenobarbital-like compounds (3, 4) or polycyclic aromatic hydrocarbon compounds (5). Therefore, the possibility exists that the production of certain BP[1] phenols, for example, by the monooxygenase and the conjugation of these phenols to inert species by the UDP-glucuronosyltransferase may be highly coupled through enzyme co-induction, thereby preventing extensive metabolism of the potentially carcinogenic BP molecule by the monooxygenase system. BP, a widespread contaminant of our environment, can be metabolized to carcinogenic forms by monooxygenase activities contained in many organs of the body. Hence, it was of interest to examine in detail the structural requirements of inducer compounds and the various requirements for macromolecular syntheses during the induction of 4-methylumbelliferone UDP-glucuronosyltransferase activity in cell culture and to compare the results with inducer requirements for AHH (EC 1.14.14.2) activity (6).

[1] Abbreviations and short terms used are: BP, benzo[a]pyrene; BA, benzo[a]anthracene; MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PB, phenobarbital; AHH, aryl hydrocarbon hydroxylase; transferase, UDP-glucuronosyltransferase; 1-OH-BP (and others), 1-hydroxybenzo[a]pyrene; BP-4,5-diol (and others), the BP derivative with a *trans*-dihydrodiol in the 4,5-position; BP-1,6-Q (and others), benzo[a]pyrene-1,6-quinone; 5-OH-BA (and others), 5-hydroxybenz[a]anthracene; BA-5,6-diol (and others), the BA derivative with a *trans*-dihydrodiol in the 5,6-position; 11-OH-MC, 11-hydroxy-3-methylcholanthrene, and PBS, phosphate buffered saline.

MATERIALS AND METHODS

Materials. Tissue culture materials used in this study were obtained from the sources cited (7). BP, BA, cycloheximide, β-glucuronidase and isoproterenol were purchased from Sigma (St. Louis, MO); metabolites of BP, BA and MC were obtained from the National Cancer Institute Chemical Repository (Bethesda, MD); actinomycin D was purchased from Calbiochem (Los Angeles, CA); 4-methylumbelliferone was from Aldrich (Milwaukee, WI); MC was obtained from Eastman (Rochester, N.Y.); TCDD was a gift from Dr. Alan P. Poland, University of Wisconsin (Madison, WI); and phenobarbital was from Merck (Rahway, NJ). H-4-II-E, a rat cell line derived (8) from Reuber hepatoma H-35, was given to us by Dr. E. Brad Thompson, National Cancer Institute (Bethesda, MD).

Methods. H-4-II-E Rueber cells, stored at −80°, were returned to growth conditions in 75-mm tissue culture flask with Eagles minimal essential medium containing 10% each of fetal calf and calf sera. For short term experiments, cells were grown in 60 mm culture dishes. Water-soluble compounds were dissolved in excess using Dulbecco's phosphate buffered saline, filtered under sterile conditions, and diluted in the growth medium to the appropriate concentration. Compounds that were not readily soluble in the growth medium were initially dissolved in a minimal amount of acetone, dimethylsulfoxide or ethanol and then added to the growth medium. The amount of organic solvent never exceeded 0.5%, a level shown not to have a detectable effect on the cells by light microscopy or by protein synthesis analysis. The concentration of any polycyclic aromatic hydrocarbon compound in the growth medium was established as previously reported (7).

Treatment of cells. Cells, plated at 10^5 cells/ml and in logarithmic growth, were treated with the appropriate inducer com-

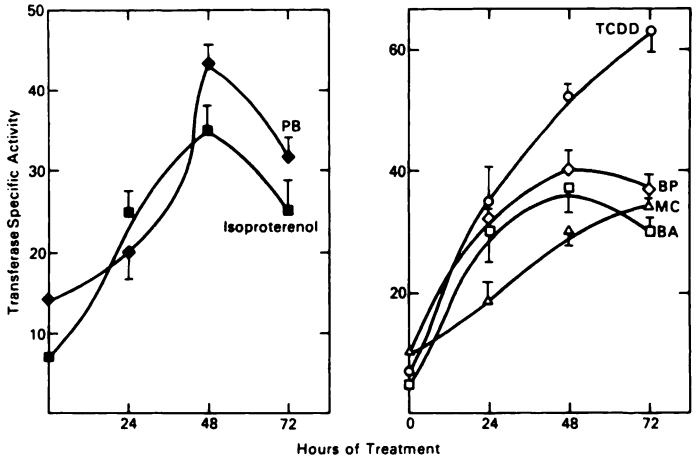

FIG. 1. *Time course of induction of UDP-glucuronosyltransferase activity in H-4-II-E cells*

Cells were treated with either 2.0 mM PB, 0.9 mM isoproterenol, 1.0 nM TCDD, 13 μM BP, 1.3 μM BA, or 4 μM MC. All cultures were changed to fresh inducer-containing medium after 48 hr of treatment. Activity was determined for the acceptor activity of 4-methylumbelliferone as described under METHODS. In this figure and in all subsequent figures, the *symbols* and *brackets* represent the mean and standard error, respectively, for at least three different determinations assayed in duplicates.

pound for varying periods of time. When cells were treated sequentially with different compounds, the cultures were washed twice with cold PBS. If treatments continued longer than 48 hr, the medium was replaced with fresh medium containing the compound(s).

Preparation of cell homogenate. Duplicate plates of cells ready for assay were washed and harvested in PBS and subsequently homogenized in PBS with a Kinematica Polytron (PCU-2 purchased from Brinkmann). Protein concentrations were determined by the method of Lowry *et al.* (9) with crystalline bovine serum albumin as standard.

UDP-glucuronosyltransferase assay.[2] The transferase activity was assayed at 24° with 4-methylumbelliferone as the acceptor substrate as previously reported (10). Fluorescence of 4-methylumbelliferone was determined on a spectrofluorometer with the activation maximum at 358 nm and the emission maximum at 450 nm. The rate of transferase activity was linearly dependent upon protein concentration between 0.150 and 0.350 mg of cell homogenate protein. One unit of transferase activity is defined as 1.0 nmole of 4-methylumbelliferone conjugated/min at 24°, calculated from the decrease in fluorescence of 4-methylumbelliferone; specific activity is expressed as units/mg of cell homogenate protein.

[2] Since the number of specific forms of UDP-glucuronosyltransferase enzymes is uncertain, in this paper transferase refers only to the activity catalyzing the glucuronidation of 4-methylumbelliferone. "Induction" denotes a net accumulation of enzyme activity following exposure of the cells to various compounds. Whether this increase represents increased protein synthesis entirely, or whether decreased degradation contributes to the net accumulation, remains to be determined.

RESULTS

Various cell culture systems have been used extensively to study the regulation and induction of several different enzyme systems (2, 11–15). Studies related to the effect of many modifier compounds on regulatory mechanisms have been made possible by the practical aspects of being able to manipulate and control chemical concentrations, exposure time, and toxicity in cell culture. These kinds of controls are not possible in *in vivo* studies. A number of tissue culture established cell lines were examined for transferase inducibility. Figure 1 shows increases in 4-methylumbelliferone UDP-glucuronosyltransferase activity in the H-4-II-E cell line by optimum concentrations of different inducer compounds. Activity after isoproterenol, phenobarbital, BP, or BA treatment peaked at

48 hr, whereas the activity after exposure to TCDD or MC continued to rise for at least 72 hr. The most potent inducer, TCDD, caused at least a 6-fold increase in activity while each of the other compounds induced transferase activity approximately 4-fold. Likewise, the UDP-glucuronosyltransferase activity in the Hepa-1 cell line was induced by phenobarbital or BA. The cell line contained a high basal level of transferase activity and less than 2-fold induction by BA (data not shown).

Since BP phenols generated by monooxygenase activity are substrates for UDP-glucuronosyltransferase activity, it was of interest to determine whether the transferase substrates are more potent inducers of the transferase activity than the native hydrocarbon. In Fig. 2, BP is shown to be a superior inducer to the seven different BP phenols, BP-9,10-diol, and BP-6,12-quinone. All the BP metabolites induced the transferase approximately 2-fold or less, except 12-OH-BP which gave slightly better than 2-fold induction. Other metabolites of BP and available metabolites of BA or MC at the optimum concentration for the native compound did not induce transferase activity (Fig. 3). It should be noted that the phenols, 3-OH- and 9-OH-BP, formed metabolically in the highest amount did not induce transferase activity.

Because the hydrocarbons appear to be the best inducers of transferase activity, as was similarly shown for AHH activity in the H-4-II-E cell line (6), the relative inducibility of transferase activity by BP and the various metabolites of BP was compared with the previously reported (6) inducibility of the AHH activity by these same compounds. Although the specific activities and the induction levels differ for the two enzymes, Fig. 4 shows that BP and most of the BP metabolites that caused the greatest enhancement of transferase activity also caused the greatest enhancement of AHH activity. The 1-OH-, 2-OH-, 5-OH-, 8-OH-, and 11-OH-BP and the BP-9,10-diol were poor inducers of either transferase or AHH activity. On the other hand, 3-OH-, 6-OH-, 7-OH-, and 9-OH-BP, BP-4,5-diol, BP-7,8-diol, BP-1,6-quinone, and BP-3,6-quinone did not induce either enzyme activity. BP-6,12-quinone was a relatively poor inducer of transferase activity but was equally as potent as BP as an inducer of AHH activity. The reasons making any BP metabolite a particularly good inducer necessarily depend on the structure of the molecule as well as the capacity of the cells to metabolize further the compound by the monooxygenase system and/or by synthesis to inert conjugates. The data in Table 1 suggest that the cells can conjugate certain substrates *in situ*.

Because phenobarbital and the hydrocar-

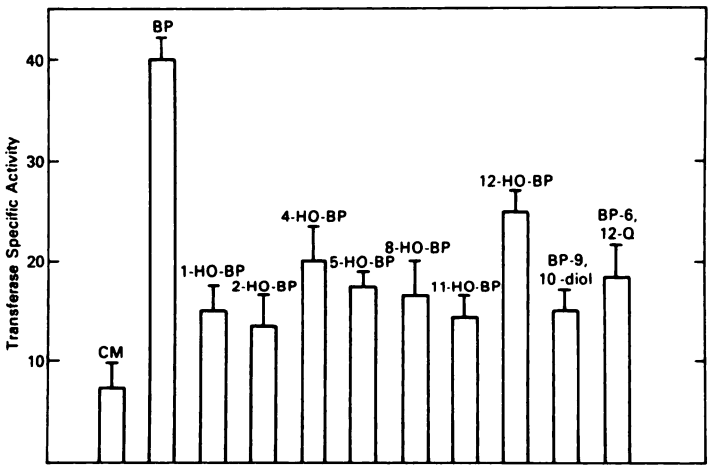

FIG. 2. *Effect of BP and various metabolities of BP on UDP-glucuronosyltransferase activity in H-4-II-E cells*

All compounds were present at 13 μM for 48 hr; CM represents control medium. Activity was determined as described under METHODS.

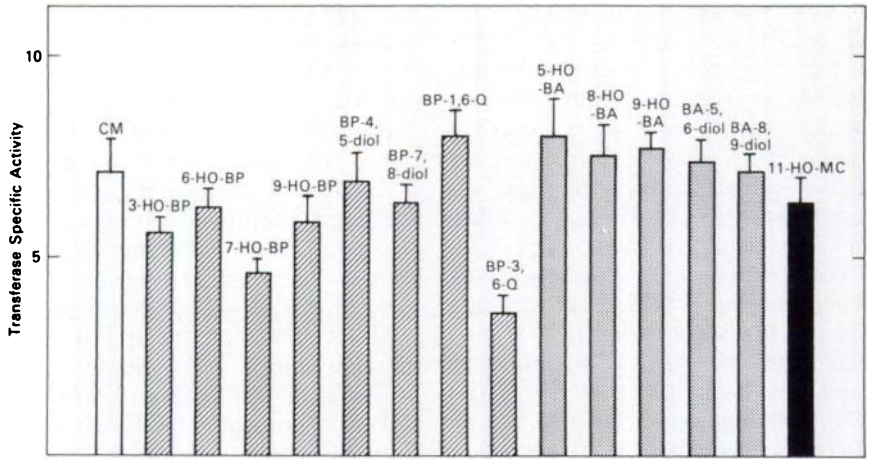

FIG. 3. *Effect of metabolities of BP, BA and MC on UDP-glucuronosyltransferase activity in H-4-II-E cells*

The metabolites were present at 13 μM for BP, 4 μM for MC and 1.3 μM for BA for 48 hr. CM represents control medium. Activity was determined as described under METHODS.

Compound	Transferase	AHH
BP	+++	+++
1-HO-BP	+	+
2-HO-BP	+	+
3-HO-BP	–	–
4-HO-BP	++	+++
5-HO-BP	+	+
6-HO-BP	–	–
7-HO-BP	–	–
8-HO-BP	+	+
9-HO-BP	–	–
11-HO-BP	+	+
12-HO-BP	++	++++
BP-4,5-Diol	–	–
BP-7,8-Diol	–	–
BP-9,10-Diol	+	+
BP-1,6-Quinone	–	–
BP-3,6-Quinone	–	–
BP-6,12-Quinone	+	+++

FIG. 4. *Relative inducibility of UDP-glucuronosyltransferase activity and AHH activity (6) in H-4-II-E cell cultures by BP and metabolites of BP at peak induction for either enzyme system*

Transferase activity was assessed from data presented in Figs. 2 and 3 of this study, and AHH activity was assessed from a previously reported study (6) using this cell line. Three plus signs (+++) represent the best inducer for the respective enzyme systems, and two plus signs (++) and one plus sign (+) represent progressively less induction. A negative (–) sign represents the lack of induction of enzyme activity.

bons represent different classes of inducers of drug-metabolizing enzymes (14, 16, 17) the effect of giving combinations of these compounds on the cells was examined for additive effects on induction of the transferase activity. Figure 5 shows that any combination of phenobarbital and an aromatic compound such as MC, BA or TCDD caused more induction than either compound alone at the optimal concentration. Combinations of two aromatic compounds did not cause additive transferase induction.

In several systems in which hormones induce a specific enzyme or protein, the increase in activity or protein level appears to be mediated, at least in part, by an increase in transcription of specific mRNA and, ultimately, an increase in translation of mRNA into specific protein (18, 19). Evidence for transcriptional and translational effects of TCDD treatment on transferase induction is shown in Fig. 6. The rise in transferase activity due to TCDD treatment was progressively inhibited by either exposure to increasing concentrations of actinomycin D or cycloheximide. Actinomycin D or cycloheximide at the concentrations used did not cause morphological damage to the cells by light microscopy

TABLE 1

Conjugation of 4-methylumbelliferone in cultured H-4-II-E cells which are controls or TCDD-induced

H-4-II-E cells were either control cultures or induced with 1.0 nM TCDD for 72 hr. To medium only, control or TCDD-induced cells, either 0.046 mg/ml of 4-methylumbelliferone (MU) or 4-methylumbelliferyl-β-D-glucuronide (MUG) was added for 5 hr. One ml of MU- or MUG-exposed medium was adjusted to pH 4.6 with 0.1 M acetate buffer pH 4.6 and treated with 0.5 mg of β-glucuronidase for 30 min at 37°; 4MU fluorescence was determined as described under METHODS. Loss of fluorescence indicates conjugation of 4MU, and restoration of fluorescence by β-glucuronidase activity confirms the existence of a β-glucuronide.

	4-Methylumbelliferone fluorescence/cell protein
4MU Conjugation in Cell Culture	
Medium alone + 4MU	55/56
Control cells + 4MU	44/43
TCDD-induced cells + 4MU	36/36
In Vitro Hydrolysis of MUG Formed in Cell Culture	
Medium + 4MU + β-glucuronidase	52/52
Control cells + 4MU + β-glucuronidase	51/51
TCDD-induced cells + 4MU + β-glucuronidase	45/45
In Vitro Hydrolysis of MUG Added to Cell Cultures	
Medium + 4MUG	0
Medium + 4MUG + β-glucuronidase	54/54
Control cells + 4MUG + β-glucuronidase	52/52
TCDD-induced cells + 4MUG + β-glucuronidase	50/49

studies. Furthermore, it was possible to stabilize TCDD-induced transferase activity by actinomycin D over a 10-hr period when compared with that in control medium (Fig. 7). Cycloheximide treatment, alone or in combination with actinomycin D, did not stabilize induced activity.

Inhibition of induction by actinomycin D suggests that mRNA synthesis is required for the rise in transferase activity. It was of interest to determine whether treatment with TCDD would cause an accumulation of induction-specific RNA under conditions of reduced translation, *i.e.*, in the presence of cycloheximide. Figure 8 shows that exposure of cells to 0.4 μM actinomycin D following a 12-hr pretreatment with TCDD and 3.5 μM cycloheximide caused a rapid rise in transferase activity. This burst of activity was not seen on exposure of pretreated cells to either TCDD or control medium alone. Actinomycin D added to the second phase of the experiment would allow no further RNA synthesis to occur, but translation of induction-specific RNA synthesized during the 12-hr pretreatment period could occur. Since *in vitro* studies of Leinwand (20) indicate that actinomycin D can stimulate translation of mRNA, it is possible that the drug has a similar effect on mRNA synthesized during the treatment period with the inducer. In the absence of the increased pool size of mRNA caused by TCDD and cycloheximide pretreatment, actinomycin D was inhibitory, as is seen in Fig. 6.

The *in vitro* studies with cell homogenates prepared from induced cells suggest that there was an increase in the levels of functional UDP-glucuronosyltransferase activity. To verify that the cells were accumulating transferase activity which could function *in situ*, we added 4-methylumbelliferone to control or TCDD-induced cultures, and after 5 hr the quantity of 4-methylumbelliferyl-β-D-glucuronide synthesized by the two cultures was compared. The TCDD-induced cells conjugated approximately twice as much 4-methylumbelliferone as control cultures (Table 1). The partial restoration of fluorescence to the 5-hr incubated medium by treatment with β-glucuronidase indicates that 4-methylumbelliferyl-β-D-glucuronide was formed by the intact cells.

DISCUSSION

In this study with the Reuber H-4-II-E established cell line, the 4-methylumbelliferone UDP-glucuronosyltransferase en-

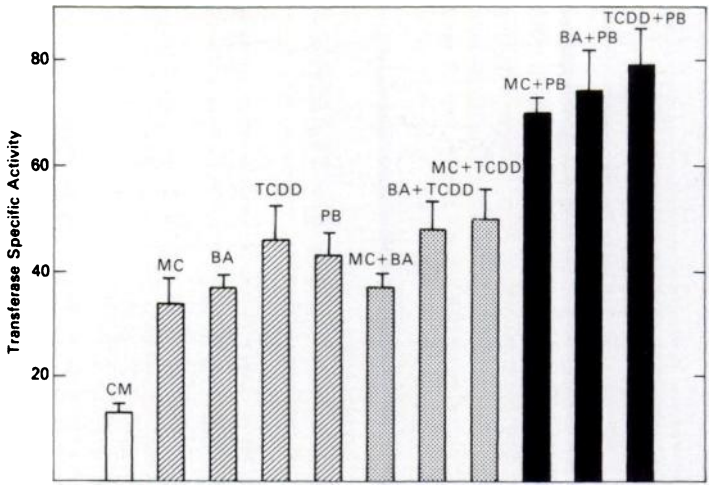

FIG. 5. *Effect of combinations of inducer compounds on UDP-glucuronosyltransferase activity in H-4-II-E cells*

Cells were exposed to 4.0 μM MC, 1.3 μM BA, 1.0 nM TCDD or 2.0 mM PB alone or in combination with each other for 48 hr. CM represents control medium. Activity was determined as described under METHODS.

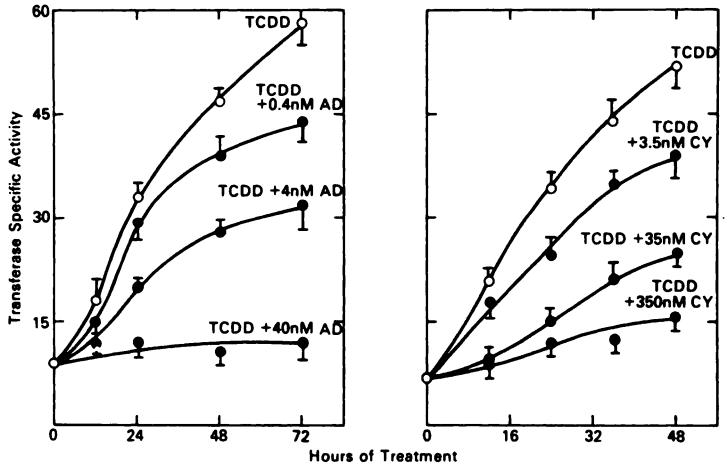

FIG. 6. *Effect of various concentrations of actinomycin D or cycloheximide on induction of UDP-glucuronosyltransferase activity by TCDD in H-4-II-E cells*

Actinomycin D or cycloheximide at the concentrations indicated was added simultaneously with TCDD. Activity was determined as described under METHODS at the times indicated.

zyme is induced several fold by either certain polycyclic aromatic compounds, phenobarbital, or isoproterenol. These compounds represent the three classes of inducers already shown to exist for induction of cytochrome P-450-dependent monooxygenase activity in fetal rat liver cell cultures (2, 14, 15) and in the H-4-II-E established cell line (7). In the established cell line, either phenobarbital- or an aromatic hydrocarbon-induced monooxygenase activity was inhibited by α-napthaflavone and not by metyrapone (21). Furthermore, Kano and Nebert[3] showed that either an optimal concentration of phenobarbital or 3-methylcholanthrene stimulated the formation of a protein band with a molecular weight of 55,000 dalton by analysis on SDS-polyacrylamide electrophoresis, and that the combination of the two inducers had an

[3] Kano, I. and D. W. Nebert, unpublished observations.

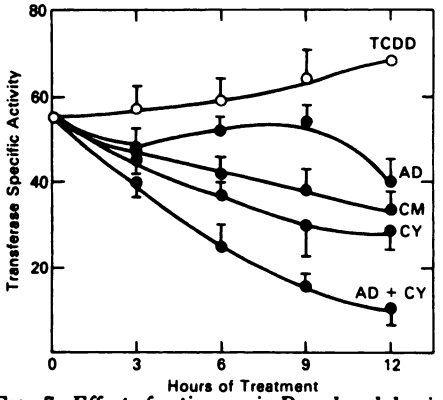

FIG. 7. *Effect of actinomycin D and cycloheximide on TCDD-induced UDP-glucuronosyltransferase activity*

All cells were exposed to TCDD for 48 hr followed by a wash and returned to TCDD-containing medium or transferred to control medium or medium containing 0.4 μM actinomycin D alone or 3.5 μM cycloheximide alone or a combination of the two inhibitors. Cells were assayed as described under METHODS at the times indicated.

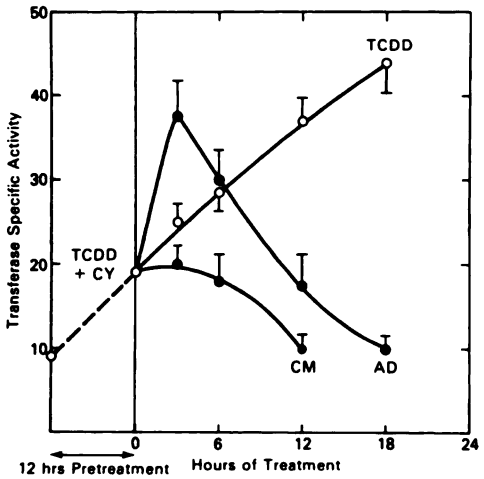

FIG. 8. *The effect of actinomycin D or TCDD on UDP-glucuronosyltransferase activity in H-4-II-E cells pretreated with TCDD and cycloheximide*

Cells were exposed to 1.0 nM TCDD and 3.5 μM cycloheximide for 12 hr, washed with control medium, and returned to TCDD alone or transferred to 0.4 μM actinomycin D or control medium (CM). Activity was determined as described under METHODS at the indicated times.

additive effect on this same protein band. The data suggest that in cell culture the inducers are not stimulating the formation of separate cytochrome P-450 as is known to occur *in vivo*, but each inducer is affecting separate steps involved in the synthesis of a single cytochrome P-450. The additive effects on transferase induction by an aromatic compound and phenobarbital, but not by two aromatic compounds, indicate that the aromatic compounds and phenobarbital, similarly, are acting at different levels to elicit induction of transferase activity in cell culture. Whether the additive effects on measured transferase activity represent two separate transferase activities cannot be determined with the present level of analyses of this class of enzymes. These additive effects of two inducers on the induction of either a single monooxygenase or transferase activity could result from the two inducers positively affecting different steps in either protein synthesis, mRNA, and/or protein degradation.

Further, if one compares the potency of the native aromatic compounds and their metabolites as inducers of transferase activity with the potency of these same compounds used in a previous study concerning induction of AHH activity (6) in H-4-II-E cells, it becomes apparent that each compound is affecting both enzyme systems in parallel, *i.e.*, substrates of the transferase such as phenolic compounds do not preferentially induce transferase activity. Moreover, the level of induction of AHH activity is always higher than the level of induction of transferase activity. The parallel in inducer requirements for transferase and AHH activities suggests that the induction processes for these two drug-metabolizing enzymes are coordinated through a common mediator, at least in the case of the aromatic compounds.

Since a cytosolic, high affinity aromatic hydrocarbon receptor has been demonstrated in the H-4-II-E cell line (22), it is likely that both monooxygenase and transferase induction processes are mediated through this receptor. Evidence for a receptor for TCDD also has been demonstrated by the uptake of radiolabeled TCDD by liver of a hydrocarbon-responsive mouse strain and the lack of the uptake by the liver of a hydrocarbon-nonresponsive strain (23). If induction of many enzymes or organ-specific proteins by steroid hormones

can serve as a model, one might expect to find a similar sequence of events for induction of drug-metabolizing enzymes by aromatic hydrocarbon compounds. Specifically, steroid hormones are known to enter target cells and to bind avidly to high affinity receptor proteins to form inducer-receptor complexes which undergo activation and translocation into the nucleus. Inducer-receptor complexes translocated to the nucleus are shown to initiate specific mRNA synthesis and ultimately increased mRNA translation into specific protein. Thus, the co-induction of the AHH and transferase enzyme systems by a single inducer suggests that a single type of cytosolic inducer-receptor complex may form and interact with different regions of chromatin with different affinities. This would ultimately allow induction of both enzymes—but such a process could involve different rates and different magnitudes. Such effects of an estrogen-receptor complex on induction of two different organ-specific proteins of the oviduct have been demonstrated in studies by Mulvihill *et al.* (24).

Although most aspects of this model have not been demonstrated for the aromatic hydrocarbon induction of drug-metabolizing enzymes, the capacity of actinomycin D to increase the appearance of transferase activity (in cells previously exposed to TCDD and sufficient cycloheximide to block partially translational activity) indicates that mRNA synthesis is a critical event in the induction process for transferase. This stimulatory action of actinomycin D—when added after prior exposure to the inducer, and compared with its inhibitory action when added with the inducer—suggests that transcriptional events are initially required for increased manifestation of transferase activity by inducer compounds. In contrast, the inhibitory action of cycloheximide on transferase activity, whether added simultaneously with the inducer or to cells already TCDD-induced, suggests that the induction process requires protein synthesis continuously. The requirements for both RNA synthesis initially and protein synthesis continuously for the induction of either AHH activity (14, 15) or the transferase activity may be taken as evidence that critical transcriptional and translational events involved are most likely initiated by the interaction of the hydrocarbon inducer-receptor complex at different chromatin sites in inducible cells.

On the other hand, mediation of both induction processes by a single compound may result from a direct action of the inducer compound and not through the high affinity, cytosolic receptor detected in the studies with TCDD (22, 23). Since induction processes in these studies respond almost always in parallel, it is likely that a common intervening entity exists to explain the coupled response. It is also possible that inducer compounds act directly to increase the level of a secondary messenger molecule which will affect both AHH and transferase activities. Since both induction processes require RNA and protein synthesis, any common mediator must necessarily act at the level of the genome and not as enzyme activators.

A specific cytosolic receptor for phenobarbital has not been demonstrated in cell culture or in *in vitro* studies. Since phenobarbital does not displace specific binding of TCDD to the aromatic hydrocarbon cytosolic receptor from C57BL/6N mice,[4] it is assumed that phenobarbital induction is mediated by a separate receptor or mechanism.

Glucocorticoid induction of UDP-glucuronosyltransferase activity in organ cultures prepared from rat fetal liver has been shown (25) to require leucine incorporation into protein. Thus, data presented here and previously reported (25) indicate that *de novo* synthesis is at least partly responsible for the net accumulation of transferase activity.

The significance of coordinated regulation of monooxygenase and certain UDP-glucuronosyltransferase activities (10) apparently through a common entity is not obvious, especially if one considers one other crucial membrane-bound drug-metabolizing enzyme, epoxide hydratase, which is not coordinately induced with

[4] Okey, A., G. P. Bondy, M. E. Mason, G. P. Kahl, H. J. Eisen, T. M. Guenthner and D. W. Nebert, unpublished observations.

monooxygenase activity (26). It would be of interest to compare co-induction of monooxygenase and UDP-glucuronosyltransferase activities with the production and conjugation of metabolites of the toxic and carcinogenic BP molecule in organs with low transferase activity, because certain major biochemically formed BP phenols are known to be potentially mutagenic (27, 28) and DNA binding species (29, 30).

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