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Microsomal Aryl Hydrocarbon Hydroxylase in Rat Adrenal: Regulation by ACTH but not by Polycyclic Hydrocarbons

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SUMMARY

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Whereas total P-450 content is more than four times greater in the rat adrenal cortex mitochondria than in microsomes, aryl hydrocarbon (benzo[a]pyrene) hydroxylase (EC 1.14.14.2) activity is more than 60 times higher in adrenal cortex microsomes than in mitochondria. The rat adrenal microsomal hydroxylase activity is strongly inhibited by α-naphthoflavone *in vitro*; progesterone 21-hydroxylase is not. In rats hypophysectomized for 30 days, aryl hydrocarbon hydroxylase decreases to about 6% of control values, but progesterone 21-hydroxylase falls to about 38% of that in sham-operated control animals. Hypophysectomy causes a striking decrease in an electrophoretic band estimated to be about 57,000 daltons. Aryl hydrocarbon hydroxylase specific activity and this electrophoretic band are restored to normal levels in adrenal microsomes of hypophysectomized rats that have received exogenous ACTH treatment. Aryl hydrocarbon hydroxylase in adrenal microsomes is not induced, however, in the hypophysectomized or intact rat by 3-methylcholanthrene or high doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. These data show that aryl hydrocarbon hydroxylase and progesterone 21-hydroxylase may be associated with different forms of adrenal microsomal P-450. It is of interest that aryl hydrocarbon hydroxylase in the adrenal of the untreated rat is similar (in sensitivity to α-naphthoflavone and in the presumed association with the 57,000-dalton apoprotein subunit on electrophoresis) to polycyclic aromatic compound-induced aryl hydrocarbon hydroxylase and its associated cytochrome P_1-450 in rat liver. The regulation of the adrenal hydroxylase by the large polypeptide hormone ACTH and the lack of inducibility by polycyclic aromatic compounds, however, are characteristics distinctly different from those of the P_1-450-associated hepatic enzyme.

INTRODUCTION

Cytochrome P-450-dependent monooxygenase activities have been discovered and studied in a large number of organs and tissues. Hepatic monooxygenases have attracted the most attention, since the liver

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contains the greatest quantity of these enzymes and is considered the dominant site of metabolism of drugs and other xenobiotics. "Extrahepatic" sites of monooxygenase activity have also become of interest. To date, monooxygenase activities have been reported in kidney, lung, skin, lymph nodes, intestine, adrenal, spleen, ovary, testis, mammary gland, bone marrow, brain, and eye (1–12). Although the levels of monooxygenase activity are low in these other organs when compared with liver, their presence may be of significant pharmacological consequence because of their capacity to: (i) metabolize immediately a compound as it enters the body and before it enters the circulation or reaches the liver; (ii) produce metabolites qualitatively different from those formed by the liver; and (iii) form beneficial or noxious metabolites directly at a subcellular site where those metabolites may express locally some specific pharmacodynamic or toxic activity.

A property of the monooxygenases that is of great biological significance is their susceptibility to induction by scores of drugs and environmental compounds. The response of hepatic microsomal monooxygenases to inducers has been studied in great detail. Different unique forms of cytochrome P-450, the terminal oxidase, are induced by different classes of chemical compounds. One such form[1] of the cytochrome, P_1-450, is induced by polycyclic aromatic compounds and metabolizes these inducers, as well as numerous other carcinogens and mutagens (12). The induction of cytochrome P_1-450 has also been studied in a number of extrahepatic tissues. P_1-450 is readily induced by polycyclic hydrocarbons in "portal-of-entry" tissues such as skin, lung, bowel, eye, and placenta (12, 16), but the response of this hemoprotein in other organs, specifically in endocrine tissue, is less well defined. Cytochrome P_1-450-catalyzed oxidation, measured as AHH[2] activity, is low in testicular (17) and ovarian (17, 18) tissue but is increased by polycyclic hydrocarbons.

The adrenal cortex is rich in cytochrome P-450 monooxygenase activity. In the human fetus, in fact, adrenal AHH is much greater than liver AHH specific activity (19). Adrenal mitochondrial monooxygenases catalyze the conversion of cholesterol to pregnenolone and steroid 11β- and 18β-hydroxylations (20). Adrenal microsomal monooxygenases predominantly catalyze, depending on species, the hydroxylation of progesterone in the 17α- or the 21-position (10). Oxidation of BP by whole homogenates of adrenal cortex, measured either by histofluorescence techniques (1) or by measurement of fluorescent metabolites generated *in vitro* (21, 22), has been reported. Induction of this activity by prior administration of polycyclic hydrocarbons is not, however, seen (21). The purpose of this report is to characterize further the rat adrenal AHH activity.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats approximately 8 weeks of age were used. Hypophysectomized and sham-operated rats were obtained from Hormone Assay, Inc. (Chicago, IL). The hypophysectomized rats were treated with ACTH or polycyclic hydrocarbon two to three weeks after operation.

Chemicals. [^{14}C]progesterone (45 mCi/mmole) was purchased from New England Nuclear Corp. (Boston, MA); corticotropin (Acthar gel) from Armor Pharmaceutical Co. (Phoenix, AZ); NADPH, NADH, and BP from Sigma Chemical Co. (St. Louis,

[1] Two or more polycyclic hydrocarbon-inducible forms of microsomal P-450 have been separated electrophoretically (13) and developmentally (14, 15) in the mouse, rat, and rabbit liver and in rabbit lung and kidney. The form having the higher molecular weight—about 56,000 or 57,000 daltons—is arbitrarily defined in this report as "cytochrome P_1-450" and has been shown (13–15) in all of the above-mentioned tissues to rise and fall concomitantly with rises and falls in polycyclic hydrocarbon-induced aryl hydrocarbon hydroxylase activity. Whether "P_1-450" is exactly the same among these species and among liver and various nonhepatic tissues remains to be determined.

[2] The abbreviations used are: AHH, aryl hydrocarbon (benzo[a]pyrene) hydroxylase (EC 1.14.14.2); BP, benzo[a]pyrene; MC, 3-methylcholanthrene; ANF, α-naphthoflavone; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

MO); MC from Eastman Kodak Co. (Rochester, N.Y.); ANF from Aldrich Chemical Co. (Milwaukee, WI); and TCDD was a gift of the Dow Chemical Co. (Midland, MI). All reagents for electrophoresis were purchased from BioRad Laboratories (Richmond, CA).

Treatment of animals and preparation of tissue fractions. Eight units of ACTH in a gelatin suspension were administered subcutaneously twice daily for 14 days. Animals were killed 12 hours after the final dose. MC in corn oil was administered as a single intraperitoneal dose of 200 mg/kg 40 hours prior to killing. Controls always received the vehicle alone within the same time-frame. TCDD in *p*-dioxane was administered as a single intraperitoneal dose of 40 μg/kg; control animals received *p*-dioxane alone (40 μl/kg). Animals were killed 2, 4, or 10 days after treatment.

The rats were decapitated and exsanguinated, and their adrenals or livers were removed and combined. Adrenals from 15 rats were pooled for each group, except in the case of hypophysectomized animals; in these groups adrenals from 40 rats were combined. The adrenals were trimmed of fat and in some cases dissected into glomerulosa and fasciculata-reticularis zones by the method cited (23). The adrenal glands were homogenized in 0.25 M sucrose with the use of a glass-Teflon homogenizer.

For mitochondrial fractions, the homogenates were centrifuged for 10 min at 750 × *g*. The supernatant fraction was collected and spun at 8700 × *g* for 10 min. The "mitochondrial" pellet was collected and resuspended either in 0.25 M sucrose or in Tris (0.02 M, pH 7.4)-KCl (0.15 M)-EDTA (0.001 M).

For microsomal fractions, tissue homogenates were centrifuged at 15,000 × *g* for 10 min, and the pellets were discarded. The supernatant fractions were centrifuged for 60 min at 105,000 × *g*, and the resulting "microsomes" were washed once by resuspending in Tris-KCl-EDTA and recentrifuging for 60 min at 105,000 × *g*. The washed pellets were resuspended in Tris-KCl-EDTA at an approximate protein concentration of 5 mg/ml and frozen at −80°. No deterioration of enzyme activity or cytochrome P-450 levels was observed during the experimental period.

Enzyme measurements. Cytochrome P-450 concentrations were measured by the method of Omura and Sato with use of an Aminco DW-2 spectrophotometer; an extinction coefficient of 91 cm/mM was used (24). The AHH activity was determined as previously described (25). *One unit* of activity is defined as that amount of enzyme catalyzing per min at 37° the formation of hydroxylated product causing fluorescence equivalent to that of 1 pmol of the 3-hydroxybenzo[a]pyrene recrystallized standard. Progesterone 21-hydroxylase activity was measured as previously described (26), *one unit* being defined as that amount of enzyme catalyzing per min at 37° the formation of 1 nmol of 21-hydroxyprogesterone. *Specific activity* denotes units per mg of microsomal protein. For the *in vitro* inhibitor studies, 10 μl of acetone containing the appropriate concentration of ANF, or 10 μl of acetone alone, was added to the reaction mixtures just prior to incubation. Protein concentration was determined as described (27).

Electrophoresis. SDS-polyacrylamide gel electrophoresis of microsomal proteins was carried out using a Bio-Rad slab gel apparatus as described in detail (13, 26). Stained slab gels were photographed, and individual tracks were cut out for densitometric analysis. The absorbance of stained protein bands was measured with use of a Gilford spectrophotometer and gel-scanner. The peaks were integrated with use of a Dietzgen manual planimeter. These data are all reported in the same arbitrary units.

RESULTS

Subcellular fractionation of adrenal AHH activity. Although the mitochondria possess approximately 80% of the total adrenal cytochrome P-450 content (Table 1), AHH activity exists predominantly in the microsomal fraction. The ratio of specific AHH activity to P-450 content was 1 unit per nmol in the mitochondria and 299 units per nmol in the microsomes. Approximately

TABLE I

Localization of AHH activity in rat adrenal cortex

Tissue Fraction	AHH activity		Total P-450 concentration	AHH activity P-450
	(units/mg protein)	*(units/adrenal)*	*(nmol/mg protein)*	*(units/nmol)*
Microsomal:				
Whole cortex	257	66	0.86	299
Zona glomerulosa	60	12	0.55	109
Zona fasciculata-reticularis	194	50	0.96	202
Mitochondrial:				
Whole cortex	3.9		3.9	1.0

80% of the total adrenal microsomal AHH activity was present in the fasciculata-reticularis zone and 20% in the zona glomerulosa.

Electrophoresis of adrenal P-450 subunits. An electrophoretic band of 57,000 molecular weight (Fig. 1) was present in both MC-treated rat liver and untreated rat adrenal microsomes. The increase and decrease in this band from rat liver microsomes is associated with the rise and fall, respectively, of MC-induced AHH activity and is therefore believed to be the subunit of cytochrome P_1-450 (13).

Preferential inhibition in vitro by ANF. ANF inhibits preferentially MC-induced AHH activity and often causes no inhibition or even enhancement of control AHH activity (28, *reviewed in ref.* 12). ANF was added to adrenal microsomes *in vitro*, and monooxygenase activities were measured (Fig. 2). In the presence of 500 μM ANF, adrenal AHH activity was inhibited about 60%, whereas progesterone 21-hydroxylase activity was only blocked about 12%. This amount of inhibition of adrenal AHH activity is at least 10 times less than the degree of inhibition of the liver enzyme; 50 μM ANF blocks about 67% of MC-induced hepatic AHH activity (28),

Effect of ACTH on adrenal AHH. When endogenous ACTH levels are lowered by hypophysectomy, it has long been known (29) that adrenal steroid hydroxylase activities are diminished. As can be seen in Table 2, 16 days after hypophysectomy both the total P-450 content and AHH levels decreased to about 20% of the levels seen in sham-operated controls. Thirty days following hypophysectomy, total P-450 content was 24%, specific AHH activity was 6%, and specific progesterone 21-hydroxylase activity was 38% of sham-operated controls. The electrophoretograms shown in Figure 3 correspond to these measurements. Tracks 2 and 3 represent adrenal microsomes from normal and sham-operated rats, respectively. Track 4 represents rats hypophysectomized for 30 days. Note

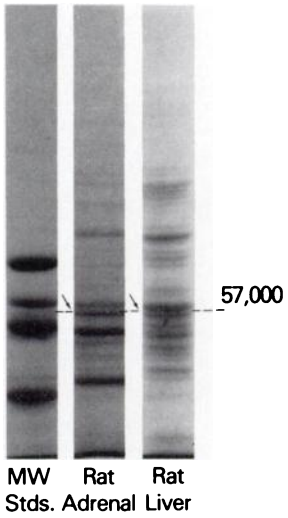

FIG. 1. *Electrophoretograms of untreated rat adrenal and MC-treated rat liver microsomes*

Fifty μg of microsomal protein were applied to each track, electrophoresed and stained as described previously (13, 26). In this figure and in subsequent figures, electrophoretic migration is from *top* to *bottom*; Track 1 contains molecular weight standards (MW Stds.) of 40,000-, 53,000-, 58,000-, and 69,000-dalton proteins. The apoprotein band having an estimated molecular weight of 57,000 is indicated by the *dashed lines* and *arrows*.

that the 57,000-dalton band has disappeared. Another electrophoretic band of slightly less molecular weight also decreased with hypophysectomy and has been recently suggested (26) to represent the progesterone 21-hydroxylase activity.

Reversal of the effects of hypophysectomy by the administration of exogenous ACTH resulted in enhancement of AHH activity and of the 57,000-dalton apoprotein band. When ACTH had been given daily for 14 days beginning 16 days after hypophysectomy, AHH activity returned to 85% of the values seen in sham-operated controls (Table 2). The 57,000-dalton band also returned, as is evident in Track 5 of Figure 3.

The observation that AHH activity is diminished by hypophysectomy and restored in the presence of ACTH prompts speculation as to the inducibility of this

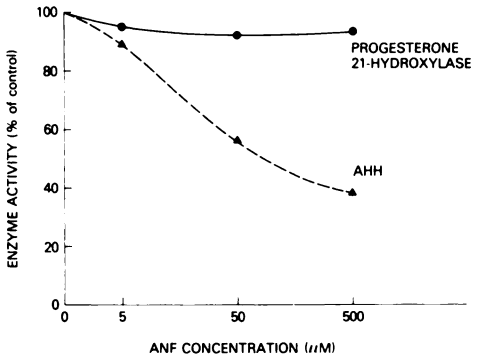

FIG. 2. *Effect of ANF* in vitro *on monooxygenase activities of rat adrenal microsomes*

Incubation conditions are described under MATERIALS AND METHODS

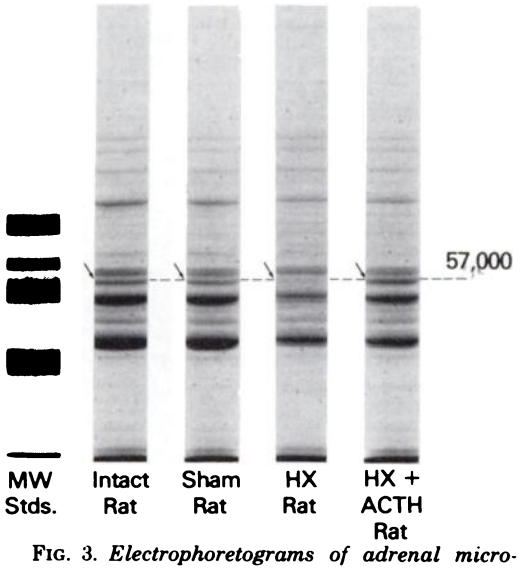

FIG. 3. *Electrophoretograms of adrenal microsomal protein from intact, sham-operated, or hypophysectomized rats, and hypophysectomized rats treated with ACTH*

Seventy-five μg of microsomal protein was applied to each well. Samples were treated the same as intact rats which were not operated upon. Sham rats were subjected to sham hypophysectomy. Hx rats were hypophysectomized 30 days before killing. Hx + ACTH rats were hypophysectomized 30 days before killing and treated with 8 units of ACTH twice daily for the last 14 days before killing. The 57,000 molecular weight apoprotein band is indicated by the *dashed lines* and *arrows*.

TABLE 2

Effects of hypophysectomy, ACTH, or MC or microsomal monooxygenase activities in rat adrenal

Intact, sham-operated, or hypophysectomized (Hx) rats were kept for 16 or 30 days before killing. Some hypophysectomized rats were treated with ACTH twice daily for 14 days, or once with MC (see MATERIALS AND METHODS).

Treatment	Total P-450 concentration	Specific AHH activity	Specific progesterone 21-hydroxylase activity	AHH activity/P-450
	(nmol/mg protein)	*(units/mg protein)*	*(units/mg protein)*	*(units/nmol)*
Intact	0.86	257	5.25	299
Sham	0.95	274	7.37	288
Hx (16 days)	0.18	56		304
Hx (30 days)	0.23	17	2.81	74
Hx (30 days); ACTH (14 days)	0.70	234	7.50	334
Hx (16 days); MC (2 days)	0.18	52		283

enzyme in the adrenal by exogenous compounds. MC was therefore administered to hypophysectomized rats, and no induction of AHH activity (Table 2) or increase in the 57,000-dalton apoprotein (Fig. 4) was seen. The lack of AHH induction in the adrenal by MC has been known for more than 10 years (21, 30). TCDD, a highly potent inducer of AHH activity in many systems (31), was administered in a high dose to intact rats 2, 4, or 10 days before killing (Table 3). Adrenal AHH activity was not increased in any of these groups.

Densitometric measurement of the 57,000-dalton apoprotein band (Fig. 5) shows that the density of this band correlates well with AHH activity among the various experimental groups. After hypophysectomy the density of this band fell to about 3.2% of control and returned to about 86% of control values after 16 days of ACTH treatment.

DISCUSSION

The fact that an adrenal microsomal monooxygenase (or monooxygenases) metabolizes BP is perhaps not in itself particularly remarkable, because BP has a chemical structure somewhat similar to that of

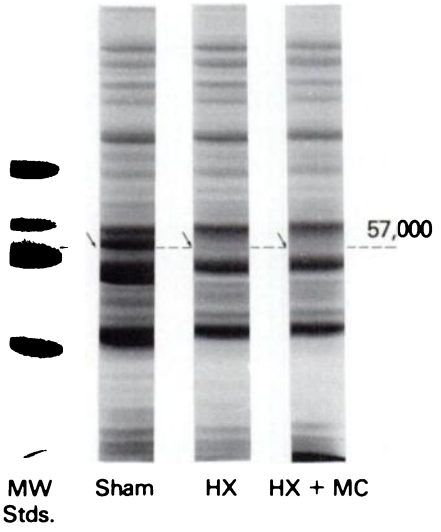

FIG. 4. *Electrophoretogram of adrenal microsomal protein from sham-operated or hypophysectomized (Hx) rats and hypophysectomized rats treated with MC (Hx + MC)*

One hundred μg of microsomal protein were applied to each well. Hx rats were hypophysectomized 16 days before sacrifice. Hx + MC rats were hypophysectomized 16 days before killing and treated once 40 hours before killing with MC. The 57,000-dalton apoprotein band is indicated by the *dashed lines* and *arrows*.

TABLE 3

Effect of TCDD on monooxygenase activities in rat adrenal microsomes

Each value represents adrenal glands combined from 15 rats.

Days after single dose of TCDD	Specific AHH activity	Total P-450 concentration	Specific progesterone 21-hydroxylase activity
	(units/mg protein)	*(nmol/mg protein)*	*(units/mg protein)*
0 (*p*-dioxane alone)[a]	260	0.90	7.52
2	250	0.99	8.03
4	235	1.06	8.77
10	205	1.02	8.05

[a] Vehicle given intraperitoneally 2 days before assay.

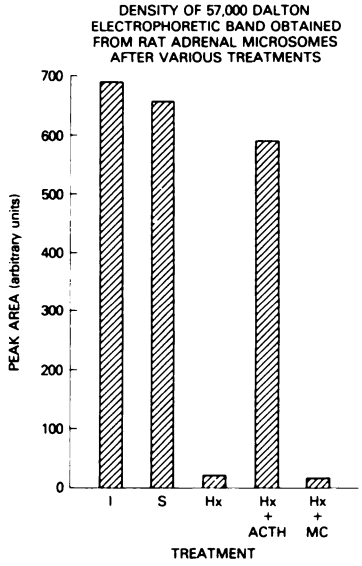

FIG. 5. *Densitometric values (in arbitrary units of area) of 57,000-dalton electrophoretic band obtained from rat adrenal microsomes after various treatments*

Electrophoretic gels described in Figures 3 and 4 were cut into strips, and the optical density of the stained protein bands was measured as described under MATERIALS AND METHODS. *I*, intact rat; *S*, sham-operated; *Hx*, hypophysectomy 30 days before killing; *Hx + ACTH*, hypophysectomy 30 days before assay and ACTH treatment daily for the final 14 days before assay; *Hx + MC*, hypophysectomy 16 days and MC treatment 40 hours before killing.

certain steroids and the adrenal monooxygenases utilize a variety of steroids as substrates. Many of the various forms of P-450 are of course known to have overlapping substrate specificities (32–34). The evidence presented here suggests, however, that the adrenal microsomal enzyme responsible for AHH activity is a distinct form of cytochrome P-450 different from the previously characterized adrenal microsomal P-450 that is responsible for progesterone 21-hydroxylation. Furthermore, this polycyclic hydrocarbon-metabolizing form of cytochrome P-450 has at least two characteristics (preferential inhibition by ANF and similar molecular weight of subunit) in common with hepatic cytochrome P_1-450 and may thus be an adrenal form of this liver enzyme. It should be emphasized that all evidence associating adrenal AHH activity with hepatic P_1-450 in this report is indirect. Cytochrome P_1-450—whether in the liver, in extrahepatic tissues, or in tissue culture—is preferentially inhibited *in vitro* by ANF (12, 28, 35). This inhibitor appears to be quite specific for polycyclic hydrocarbon-induced monooxygenase activities, since many P-450-dependent (rather than P_1-450-dependent) oxidations are not preferentially blocked by this compound (14, 35, 36). In the adrenal, AHH activity is readily inhibited by ANF but progesterone hydroxylase activity is not (Fig. 2). These data suggest that the adrenal polycyclic hydrocarbon-metabolizing enzyme is similar to the hepatic MC-inducible enzyme, but is distinct from the adrenal enzyme that hydroxylates progesterone in the 21-position.

SDS-polyacrylamide gel electrophoresis of rat adrenal microsomes shows the presence of a 57,000-dalton subunit which appears to be the same size as the 57,000-dalton apoprotein of hepatic cytochrome P_1-450 (Fig. 3). Although the appearance of this band is not sufficient evidence to prove that it is the apoprotein of a cytochrome P_1-450, the susceptibility of this protein to alteration by various hormonal states strongly suggests its association with ANF-sensitive AHH activity. Thirty days after hypophysectomy, AHH activity falls to 6% of control values; concomitantly, the 57,000-dalton band virtually disappears from the electrophoretogram. It should be noted that AHH activity is calculated per milligram of microsomal protein so that while the entire adrenal atrophies in the absence of ACTH, AHH activity is more labile than total adrenal protein and falls to a much lower level. Furthermore, AHH activity is more labile than total microsomal P-450 levels. In the control animal, the ratio of AHH to total P-450 is 288; in the 30-day hypophysectomized animal it is 74. AHH activity continues to fall, so that the activity at 30 days is at least three times lower than that at 16 days after hypophysectomy. Total P-450 levels at 30 days are not lower than those 16 days after hypophysectomy (Table 2). Progesterone 21-hydroxylase activity also falls after hypophysectomy, to 38% of control values. AHH activity is diminished much more (to 6% of control) than progesterone hydroxylase activity (to 38% of control); these data indicate further that these two activities do not reflect the same enzyme. Perhaps of interest and relevant to the present study, we have also found (unpublished data) that microsomes from dog adrenals—which have a much lower specific AHH activity (7.5 pmol/min/mg as compared with 260 pmol/min/mg for rat adrenals)–exhibited very little, if any, of this 57,000-dalton electrophoretic band.

One might argue that the similarities between adrenal AHH and hepatic P_1-450-associated AHH (preferential sensitivity to ANF and the apparent identical molecular weight of the subunit after electrophoresis) might be coincidental. Antibodies to hepatic P_1-450 could be useful in testing this possibility further by attempting to block adrenal AHH activity.

Administration of ACTH to hypophysectomized animals concomitantly restores adrenal microsomal AHH activity and the 57,000-dalton electrophoretic band. Whereas adrenal AHH appears to be catalytically similar to hepatic MC-induced AHH, however, the response of adrenal AHH to polycyclic aromatic inducers is distinctly different. No increase in adrenal microsomal P-450 or AHH activity was observed in hypophysectomized MC-treated rats (Table 2). Moreover, examination of an

electrophoretogram of adrenal microsomes from these animals showed no increase in density of any of the protein bands (Fig. 4). Though other workers have shown adrenal AHH from intact animals to be insensitive to hydrocarbon inducers (1, 21, 30), we show here that AHH-depleted adrenals from hypophysectomized rats, which may relate more directly to the "uninduced" rather than to the "ACTH-induced" state, are also insensitive to this form of chemical induction. Further, TCDD—the most potent known inducer of cytochrome P_1-450 (31)—does not induce adrenal microsomal AHH at any period between 2 and 10 days after injection. Within 10 days after TCDD treatment, the animal was visibly wasted and the beginning of necrosis in the liver and adrenal was noted.

If one compares the ratio of AHH activity to total P-450 content in microsomes, untreated rat liver and the hypophysectomized rat adrenal have similar ratios (between 70 and 80). MC-treated rat liver and the intact rat adrenal (*i.e.*, MC-induced *versus* "ACTH-induced") also have similar ratios—between 220 and 300.

The response of adrenal AHH activity to induction by ACTH but not by polycyclic hydrocarbons is the only known example of an apparent P_1-450-type cytochrome which is inducible by an endogenous hormone but not by exogenous chemicals. Furthermore, this is the first example of AHH activity inducible by a large molecular weight polypeptide (37). ACTH had been found (38) to have no effect on basal or inducible AHH activity in fetal rat primary hepatocyte cultures. In the liver, AHH levels are thought to be regulated by small molecular weight compounds (*i.e.*, 250 to 450 daltons) in a manner similar to the regulation of various enzyme levels by steroids (*discussed in detail in ref.* 12). The inducer is known to diffuse into the cytoplasm and bind with high avidity to a receptor protein, thereby initiating a series of events (pleiotypic response) which results in the increased synthesis of specific gene products. Although the AHH activity induced by ACTH in the adrenal is evidently quite similar to that induced by xenobiotics in the liver, the chain of events by which the hormone increases the AHH-associated form of P-450 must be quite different. ACTH is believed to act at the cell surface, initiating a "second messenger"-type response, and both microsomal and mitochondrial monooxygenases activities are enhanced (37). Subsequent chemical events may then stabilize adrenal AHH activity, stimulate *de novo* protein synthesis, or block the normal rate of degradation. P_1-450 induction by β-naphthoflavone and P-450 induction by phenobarbital in mouse liver are both known to represent *de novo* protein synthesis (39).

The fact that very similar "P_1-450" cytochromes are induced in different organs by very different inducers raises intriguing questions as to the normal physiological role of this enzyme in the body. Since the known substrate(s) for AHH are nonphysiological, one hypothesis (40) of the origin of this enzyme has been that exogenous substrates combine with certain receptor proteins in a manner similar to the combination of the normal physiological ligands (steroids?) with their receptors. This combination then could lead to induction of monooxygenases normally under steroidal control. Mutations in the genes coding for these enzymes might result in slightly altered monooxygenases, which could readily metabolize the foreign compound, thus giving the organism the evolutionary advantage of detoxification. However, the presence of AHH in the adrenal, an organ which normally does not encounter foreign compounds as do such "portal of entry" organs as the skin, lung, eye, or intestine, suggests that this enzyme may have developed independently of the selective pressures applied by toxic compounds. The fact that adrenal AHH is inducible by ACTH suggests that the adrenal enzyme may not have developed as a response to toxic compounds but rather is a normal constitutive enzyme which somehow has become inducible in certain organs by exogenous compounds.

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REFERENCES

1. Wattenberg, L. W. & Leong, J. L. (1962) Histochemical demonstration of reduced pyridine nucleotide dependent polycyclic hydrocarbon metabolizing systems. *J. Histochem. Cytochem.*, **10,** 412–420.
2. Wattenberg, L. W., Leong, J. L. & Strand, P. J. (1962) Benzpyrene hydroxylase activity in the gastrointestinal tract. *Cancer Res.*, **22,** 1120–1125.
3. Kato, R. (1966) Possible role of P-450 in the oxidation of drugs in liver microsomes. *J. Biochem.*, **59,** 574–583.
4. Uhleke, H. & Greim, H. (1968) Stimulierung der oxydation von fremdstoffen in nierenmicrosomen durch phenobarbital. *Arch. Pharmakol. Expt. Pathol.*, **261,** 152–161.
5. Ellin, A., Jakobsson, S. V., Schenkman, J. B. & Orrenius, S. (1972) Cytochrome P_{450k} of rat kidney cortex microsomes; its involvement in fatty acid ω- and (ω-1) hydroxylation. *Arch. Biochem. Biophys.*, **150,** 64–69.
6. Hook, G. E. R., Bend, J. R., Hoel, D., Fouts, J. R. & Gram, T. E. (1972) Preparation of lung microsomes and a comparison of the distribution of enzymes between subcellular fractions of rabbit lung and liver. *J. Pharmacol. Exp. Ther.*, **182,** 474–480.
7. Schachter, B. A., Nelson, E. B., Marver, H. S. & Masters, B. S. S. (1972) Immunochemical evidence for an association of heme oxygenase with the microsomal electron transport system. *J. Biol. Chem.*, **247,** 3601–3607.
8. Benedict, W. F., Considine, N. & Nebert, D. W. (1973) Genetic differences in aryl hydrocarbon hydroxylase induction and benzo[a]pyrene-produced tumorigenesis in the mouse. *Mol. Pharmacol.*, **9,** 266–277.
9. Nebert, D, W., Levitt, R. C., Jensen, N. M., Lambert, G. H. & Felton, J. S. (1977) Birth defects and aplastic anemia; differences in polycyclic hydrocarbon toxicity associated with the *Ah* locus. *Arch. Toxicol.*, **39,** 109–132.
10. Mattison, D. R. & Thorgeirsson, S. S. (1977) Genetic differences in mouse metabolism of benzo[a]pyrene and oocyte toxicity. *Biochem. Pharmacol.*, **26,** 909–912.
11. Mason, J. I., Estabrook, R. W. & Purvis, J. L. (1973) Testicular cytochrome P-450 and iron-sulfur protein as related to steroid metabolism. *Ann. N.Y. Acad. Sci.*, **212,** 406–419.
12. Nebert, D. W., Atlas, S. A., Guenthner, T. M. & Kouri, R. E. (1978) The *Ah* locus; Genetic regulation of the enzymes which metabolize polycyclic hydrocarbons and the risk for cancer, in *Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment,* (Ts'o, P. O. P. and H. V. Gelboin, eds.). Academic Press, New York, 345–390.
13. Boobis, A. R., Nebert, D. W. & Felton, J. S. (1977) Comparison of β-naphthoflavone and 3-methylcholanthrene as inducers of hepatic cytochrome(s) P-448 and aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity. *Mol. Pharmacol.*, **13,** 259–268.
14. Atlas, S. A., Boobis, A. R., Felton, J. S., Thorgeirsson, S. S. & Nebert, D. W. (1977) Ontogenetic expression of polycyclic aromatic compound-inducible monooxygenase activities and forms of cytochrome P-450 in the rabbit; evidence for temporal control and organ specificity of two genetic regulatory systems. *J. Biol. Chem.*, **252,** 4712–4721.
15. Guenthner, T. M. & Nebert, D. W. (1978) Evidence in rat and mouse liver for temporal control of two polycyclic aromatic-inducible forms of cytochrome P-450. *Eur. J. Biochem.*, **91,** 449–456.
16. Conney, A. H. (1971) Environmental factors influencing drug metabolism, in *Fundamentals of Drug Metabolism and Drug Disposition* (LaDu, B. N., H. G. Mandel and E. L. Way, eds.). Williams and Wilkins Co., Baltimore, 253–269.
17. Stripp, B., Menard, R. H. & Gillette, J. R. (1974) Effect of chronic treatment with phenobarbital or 3-methylcholanthrene on the male reproductive system in rats. *Life Sci.*, **14,** 2121–2130.
18. Mattison, D. R. & Thorgeirsson, S. S. (1978) Gonadal aryl hydrocarbon hydroxylase in rats and mice. *Cancer Res.*, **38,** 1368–1373.
19. Pelkonen, O. & Kärki, N. T. (1973) Drug metabolism in human fetal tissues. *Life Sci.*, **13,** 1163–1180.
20. Wickramasinghe, R. H. (1975) Biological aspects of cytochrome P450 and associated hydroxylation reactions. *Enzyme*, **19,** 348–376.
21. Dao, T. L. & Yogo, H. (1964) Effects of polynuclear aromatic hydrocarbons on benzpyrene hydroxylase activity in rats. *Proc. Soc. Exp. Biol. Med.*, **116,** 1048–1051.
22. Feuer, G., Sosa-Lucero, J. C., Lumb, G. & Moddel, G. (1971) Failure of various drugs to induce drug-metabolizing enzymes in extrahepatic tissues of the rat. *Toxicol. Appl. Pharmacol.*, **19,** 579–585.
23. Giroud, C. J. P., Stachenko, J. & Venning, E. H. (1956) Secretion of aldosterone by the zona glomerulosa of rat adrenal glands incubated *in vitro*. *Proc. Soc. Exp. Biol. Med.*, **92,** 154–158.
24. Omura, T. & Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.*, **239,** 2379–2385.
25. Nebert, D. W. & Gielen, J. E. (1972) Genetic regulation of aryl hydrocarbon hydroxylase in-

duction in the mouse. *Fed. Proc.*, **31,** 1315–1325.
26. Menard, R. H., Guenthner, T. M., Kon, H. & Gillette, J. R. (1978) Studies on the destruction of adrenal and testicular cytochrome P-450 by spironolactone; requirement for the 7-α-thio group in evidence for the loss of the heme and apoprotein of cytochrome P-450. *J. Biol. Chem.*, **254,** 1726–1734
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193,** 265–275.
28. Goujon, F. M., Nebert, D. W. & Gielen, J. E. (1972) Genetic expression of arylhydrocarbon hydroxylase induction; IV. Interaction of various compounds with different forms of cytochrome P-450 and the effect on benzo[a]pyrene metabolism *in vitro*. *Mol. Pharmacol.*, **8,** 667–680.
29. Purvis, J. L., Canick, J. A., Mason, J. I., Estabrook, R. W. & McCarthy, J. L. (1973) Lifetime of adrenal cytochrome P-450 as influenced by ACTH. *Ann. N. Y. Acad. Sci.*, **212,** 319–343.
30. Nebert, D. W. & Gelboin, H. V. (1969) The *in vivo* and *in vitro* induction of aryl hydrocarbon hydroxylase in mammalian cells of different species, tissues, strains, and developmental and hormonal states. *Arch. Biochem. Biophys.*, **134,** 76–89.
31. Poland, A. & Kende, A. (1976) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Environmental contaminant and molecular probe. *Fed. Proc.*, **35,** 2404–2411.
32. Haugen, D. A., van der Hoeven, T. A. & Coon, M. J. (1975) Purified liver microsomal cytochrome P-450. Separation and characterization of multiple forms. *J. Biol. Chem.*, **250,** 3567–3570.
33. Ryan, D., Lu, A. Y. H., West, S. & Levin, W. (1975) Multiple forms of cytochrome P-450 in phenobarbital- and 3-methylcholanthrene-treated rats. *J. Biol. Chem.*, **250,** 2157–2163.
34. Guengerich, F. P. (1977) Separation and purification of multiple forms of microsomal cytochrome P-450. Activities of different forms of cytochrome P-450 towards several compounds of environmental interest. *J. Biol. Chem.*, **252,** 3970–3979.
35. Nebert, D. W., Considine, N. & Owens, I. S. (1973) Genetic expression of aryl hydrocarbon hydroxylase induction; VI. Control of other aromatic hydrocarbon-inducible monooxygenase activities at or near the same genetic locus. *Arch. Biochem. Biophys.*, **157,** 148–159.
36. Atlas, S. A. & Nebert, D. W. (1976) Genetic association of increases in naphthalene, acetanilide, and biphenyl hydroxylations with inducible aryl hydrocarbon hydroxylase in mice. *Arch. Biochem. Biophys.*, **175,** 495–506.
37. Scott, A. P., Bloomfield, G. A., Lowry, P. J., Gilkes, J. J. H., Landon, J. & Rees, L. H. (1976) Pituitary adrenocorticotrophin and the melanocyte stimulating hormones, in *Peptide Hormones* (Parson, J. A., ed.). University Park Press, Baltimore, 247–271.
38. Gielen, J. E. & Nebert, D. W. (1972) Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture. III. Effects of various sera, hormones, biogenic amines, and other endogenous compounds on the enzyme activity. *J. Biol. Chem.*, **247,** 7591–7602.
39. Haugen, D. A., Coon, M. J. & Nebert, D. W. (1976) Induction of multiple forms of mouse liver cytochrome P-450, evidence for genetically controlled *de novo* protein synthesis in response to treatment with β-naphthoflavone or phenobarbital. *J. Biol. Chem.*, **251,** 1817–1827.
40. Nebert, D. W., Owens, I. S., Kano, I. & Guenthner, T. M. (1978) Aryl hydrocarbon hydroxylase. Evidence of receptors for induction process in cell culture, in *Advanc. Pharmacol. Therapeutics* Vol. 10, "Chemotherapy," (M. Adolphe, ed.). 185–196. Pergamon Press Ltd., Oxford, England.